

A COLOR ATLAS OF MEDICAL BACTERIOLOGY

*An overview about the identification tests and
the cultivation methods for various bacterial
Genera and species regarding teaching
laboratories isolates*

وزارة الصحة- دائرة مدينة الطب- مديرية المختبرات التعليمية
أنجز من قبل مديرية المختبرات التعليمية بالتعاون مع مختبر الصحة العام المركزي (عام 2008)

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الأهداء :

..... الى من هم عن عيوننا غائبون

والله اعلم في قلوبنا وأرواحنا ما كنون

المقدمة

حينما خلفت بأعطاء مادة البكتريولوجي العملي لدورة المختبرات (35) المقامة في المختبرات التعليمية فكرت في طباعة الفحوصات البايوكيميائية ليرجع اليها الطالب حينما يحتاجها . ثم سحنت لي الفرصة بأنزال صور هذه الفحوصات حينما توفرت كاميرا حديثة لدى احد طلبتي ففكرت بأثراء تلك الصور بمعلومات يستفاد منها البكتريولوجي اثناء عمله وذلك بكتابة بعض المعلومات التشخيصية موضحة بصور فوتوغرافية للكائنات المجهريّة المعزولة في مختبراتنا .

أود أن أؤمن جهود المستشفيات التابعة لدائرة مدينة الطب لمساعدتنا في إنجاز هذا العمل وذلك بتزويدي ببعض الأحياء المجهريّة ، وأخص بالذكر مختبر الأحياء المجهريّة في كل من مستشفى الجراحات التخصصية ، مستشفى حماية الأطفال ، معهد الأمراض الصدرية و وحدة الدورات في المختبرات التعليمية .

أشكر الدكتور هيثم أحمد الربيعي مدير المختبرات التعليمية لتشجيعه لي وكذلك الدكتور جاسم طعمة الخفاجي.

أؤمن جهود الإدارة العامة التابعة لدائرة مدينة الطب وأخص بالذكر (كادر شعبة الأمور الفنية ، شعبة المختبرات والوحدة العلمية) .

مزيد من الشكر والأمتنان لكل من ساعدني في إنجاز هذا العمل ولو بكلمة طيبة اذكر منهم (الأستاذ زهير إبراهيم ، البكتريولوجي كامل كاظم مشالي ، البكتريولوجي علي عبد الكريم حسون ، البايولوجية رويدة إسماعيل احمد ، البايولوجية زينب علي ناجي ، البايولوجية رواء أسعد سلمان ، البايولوجية ندى ساجدة ، مساعد مختبر الآء عبد العال ، البايولوجية رنا باسم ، البايولوجي حسين علي ، الدكتور علي جليل ، الأستاذ خالد مهدي صالح) .

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لدراسة اقتراحاتكم وملاحظاتكم بكل اعتزاز .

البكتريولوجية الاختصاص

ليلى عبد الكريم وحيد

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GRAM'S STAINING METHOD

- 1-fix smear by heat
2. Flood slide with **crystal-violet** solution:
Allow to act for **10-30 sec.**

Methyl-violet stain

1 percent aqueous solution of
Crystal-violet 30 parts **5** percent, solution of
sodium bicarbonate **8** parts

3. Wash off stain with water.

4. Cover with Grams **iodine 10-30 sec.**
(The solution may be stored for months at room temperature, but if a characteristic **amber colour is observed, it must be discarded**)

Iodine solution

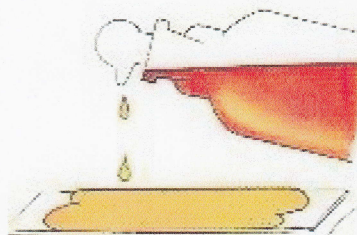
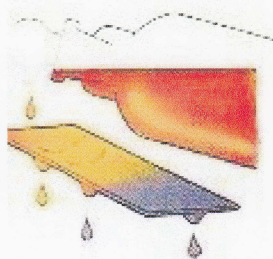
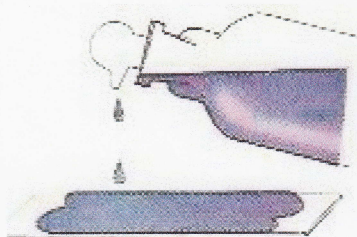
Iodine **2** gm.

Normal solution of sodium hydroxide **10** ml.

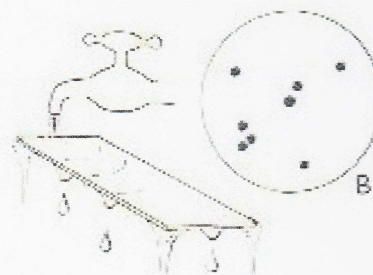
Distilled water **90** ml.

- 5- Wash with water.

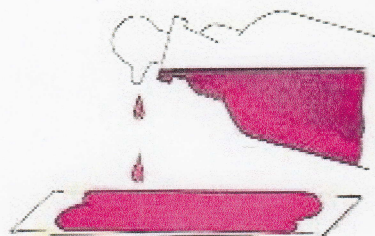
- 6- Decolorized with acetone for not more than **5 sec.** or decolorized for **10-30 sec.** with
(acetone **30** ml with alcohol **70** ml)



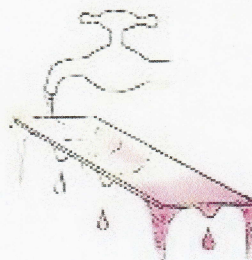
7- Wash slide immediately in water.



8- Cover for **10 – 30 sec.** with **safranin.**
(**Safranin. 2.5** gm. & **100** ml)
Ethyl alcohol **95%**)



9- Wash in water, blot and dry in air.



The inserts indicate the appearance of a mixed
Gram +ve and Gram -ve film at different stages
during staining.

A Before acetone decolorisation all organisms
appear Gram +ve.

B After acetone decolourisation those organisms
which are Gram -ve are no longer visible.

C These Grams -ve organisms are visualized
After the application of the counterstain.



ZIEHL-NEELSEN'S STAINING METHOD

- 1- Fix smear by heat
- 2- Flood slide with **carbol fuchsin**. Allow to act for **5** min. HEAT intermittently without boiling the stain.

Ziehl-Neelsen carbol fuchsin

Basic fuchsin **1g.**

Absolute alcohol **10 ml.**

Phenol solution (5 percent in water) **100 ml.**

The dye is dissolved in the alcohol and added to the phenol solution.

- 3- Wash with water.

- 4- Flood slide with **20 percent H_2SO_4** . After **1** min. wash in water and apply fresh acid.

Repeat process several times.

- 5- Wash thoroughly with water

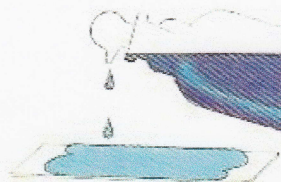
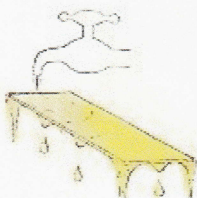
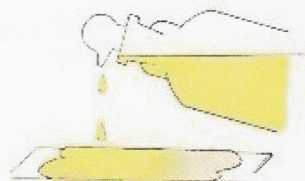
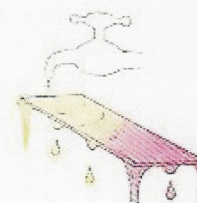
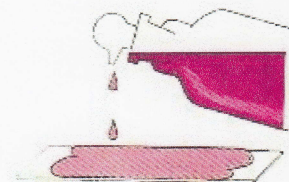
- 6- Apply **methylene blue** counterstain for **10- 30** sec.

Loeffler's methylene Blue.

Saturated solution of methylene blue in alcohol **30 ml.**

KOH (**0.01** percent in water) **100ml.**

- 7- Wash in water, blot and dry in air. Since tap water may contain saprophytic acid-fast mycobacteria it Should not be used to make up staining reagents For use in the Ziehl -Neelsen method; similarly washing of the preparation at all stages during staining should be with water non to be free from such saprophytic species.

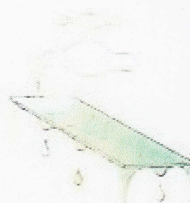


ALBERT'S STAINING METHOD

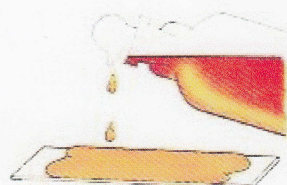
1- Apply solution 1; allow to act for 3-5 min. -



2- Wash in water.



3- Apply solution 2; allow acting for 1 min.



4- Wash and blot dry.



Solution 1

Toluidine blue 0.15 g.

Malachite green 0.2 g.

These are dissolved in 2 ml. of 95 percent alcohol and added to 100 ml. of distilled water containing 1 ml. of glacial acetic acid. Ready for use after standing for 24 hr. and being filtered.

Solution 2

Iodine 2 g.

Pottasium iodide 3 g.

Disteled water 300 ml.

Bacterial Identification Methods

I- Catalase Test

Short review:

The bacteria produce hydrogen peroxide (H_2O_2) during their aerobic respiration, and if it accumulates inside the bacterial cells, it's too toxic, so usually most bacteria (aerobic & facultative anaerobic) will utilize this enzyme to degrade H_2O_2 .

Principle:

The enzyme **catalase** mediates the breakdown of **hydrogen peroxide into oxygen and water**. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide (3% solution), and a rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

Method:

- Use a loop or sterile wooden stick to transfer a small amount of colony growth to the surface of a clean, dry glass slide.
- Place a drop of **3% hydrogen peroxide (H_2O_2)** onto the inoculum.
- Observe for the evolution of oxygen bubbles.

Expected Results:

Catalase-positive organisms (e.g., *staphylococci*, *Listeria monocytogenes*, and *corynebacterium* spp.) produce copious bubbles; catalase-negative organisms (e.g., *streptococci* and enterococci) yield no or few bubbles.

Note: some bacteria produce peroxides that slowly catalyze the breakdown of (H_2O_2) and the test may appear weakly positive (a few bubbles slowly elaborated). This reaction is not a truly positive test and is considered negative.

Notes to be observed:

- 1- Don't use media containing blood, because the red blood cells contain Catalase and it will give us a false positive test.
- 2- Always use a fresh H_2O_2 because it's unstable and check it via a control known strain of a Catalase positive one.

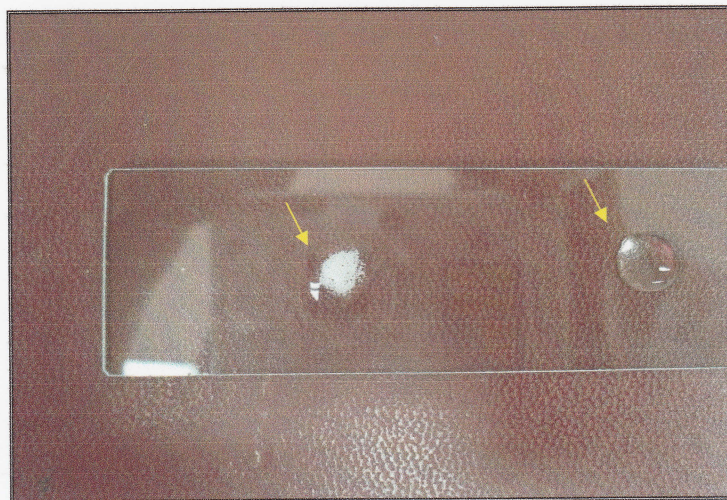


Figure (1): Revealed a positive catalase test regarding *Staphylococci* spp. Isolated from impetigo case (left) and a negative result regarding *Streptococcus* spp. Isolated from pharyngitis case. Notice the bubbles in the positive result.

II- Oxidase Test

Short review:

The electron transport chain is a sequence that yielded from the bacterial cell respiration, and this stage is mediated via the cytochrome oxidase enzyme in which it oxidize the electron transport molecule and reduce the oxygen into water.

Principle:

By the using of the oxidation substrate which is (1% **Tetramethely – P-phenylenediamine dihydrochloride**) or (4, NNNN) to indophenols, a **dark purple** - colored end product. A positive test (presence of oxidase) is indicated by the development of a **dark purple** color. Pink or no color development indicates a negative test and the absence of the enzyme.

Method:

- Moisten filter paper with the substrate (**1% tetramethyle -p-phenylenediamine dihydrochloride**) or select a commercially available paper disk that has been impregnated with the substrate.
- Use a platinum wire or wooden stick to remove a small portion of a bacterial colony (preferably not more than **24 hours** old) from the agar surface and rub the sample on the filter paper or commercial disk.
- Observe inoculated area of paper or disk for a purple **color change to deep blue within 10 seconds** (timing is critical).

****Note: oxidase reagent advisable to store in powder at 4°C since it has a very short life time when is dissolved.**

Expected Results:

positive organisms, such as *Neisseria* spp., *Brucella* spp., *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Haemophilus influenzae* turn the filter paper dark purple within **10 seconds**; negative organisms, such as Entrobactereaceae (e.g., *Escherichia coli*) remain colorless or the color of the inoculums.

Oxidase positive genus:

[1] *Pseudomonas* spp., [2] *Aeromonase* spp., [3] *Pleisomonas* spp., [4] *Vibrio* spp., [5] *Campylobacter* spp., [6] *Neisseria* spp., [7] *Moraxella* spp. , [8] *Achromobacter* spp. [9] *Rhizobium* spp. , [10] *Burkholderia* spp. , [11] *Chryseobacterium*, [12] *sphingo-bacteruim* spp., [13] *Alcaligenes* spp. , [14] *Bordetella not pertusis*

Notes to be observed:

- 1- Always test the reactivity of our reagent via well-known oxidase positive bacteria.
- 2- Always neglect any color changes to bacterial isolates outside the border of our specimen taken onto the filter paper.
- 3- Always neglect any color changes beyond **60** seconds.

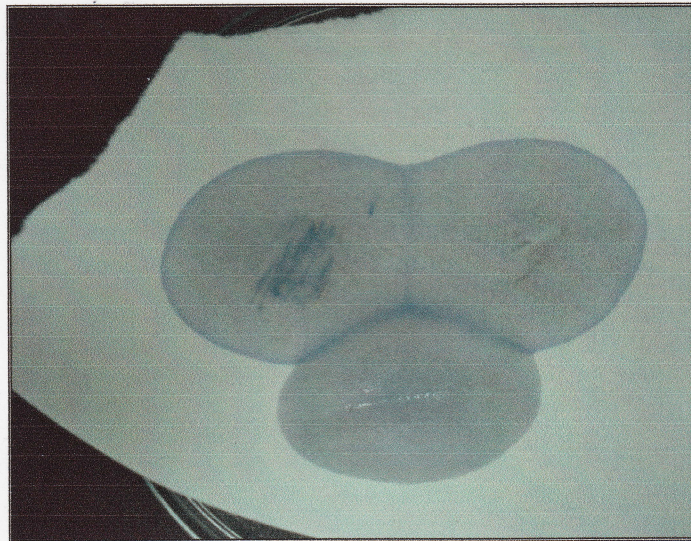


Figure (2): Shows comparison between a positive oxidase test results (left) regarding *Pseudomonas aeruginosa* isolated from chronic otitis case. Notice the purple color in the positive test result and a negative test result (right) regarding *Staphylococcus* spp. (right) plus *Streptococcus* spp. (inferiorly).

III- Indole Test

Short review:

Indole production is the “I” portion of the test IMViC tests used in enteric bacteria identification, the amino acid tryptophan could be degraded via the enzyme tryptophanase to yield ammonia (NH_3), indole, and pyruvic acid, so this enzyme will differentiate between *E.coli* from *Enterobacter aerogenes marcescens* whose don't have this enzyme.

Principle:

Indole which accumulates in the test tube is tested by a colorimetric reaction with P-dimethyl-aminobenzaldehyde.

Medium: contain Peptone (20 g), NaCl_2 (5g), D.W (1litre).

A adjust the pH to 7.4 dispense and sterilize by autoclaving at 121°C for 15 minutes

Kovac's reagent:

Amyl or isoamylalcohol **150** ml.

p- dimethyl-aminobenza-ldehyde **10** g.

Hydrochloric acid, concentrated, Hcl **50** ml.

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

Method:

- Inoculate medium and incubate for **48** h. at **37°C**. Sometimes a period of **48** h. at **37°C** may be required for optimum accumulation of indole.
- Add **0.5** ml **Kovac's reagent** and shake gently. A **red** color in the alcohol layer indicates a positive reaction.(reagents must be stored refrigerated and avoiding direct light

Notes to be observed:

- 1- Always test the reactivity of Kovac's reagent via well-known positive bacterial strain.
- 2- Look for color changes in the used alcohol surface.

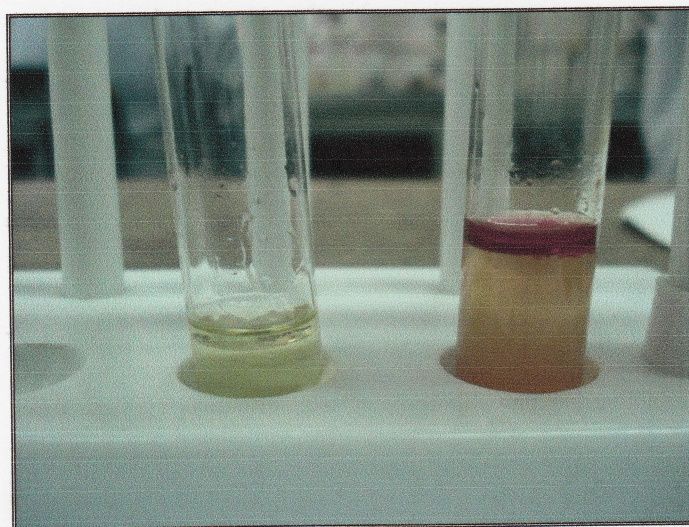


Figure (3): Shows indole [tryptophane production] positive test result with red ring regarding a growth of *E.coli* (right), and negative result regarding *Klebsiella pneumoniae*. (left).

IV- Bile Solubility Test

Short review:

The bile solubility test differentiates *streptococcus pneumoniae* (positive) from alpha-hemolytic *streptococci* (negative). Bile or solution of a bile salt, such as sodium deoxycholate lyses pneumococcal colonies.

Principle:

Lysis depends on the presence of an intracellular autolytic enzyme. Bile salts lower the surface tension between the bacterial cell membranes and the medium, thus accelerating the organisms' natural autolytic process.

Method:

- Place **1 to 2 drops of 10% sodium deoxycholate** to the side of a **young (13- to 24-hour)**, well isolated colony growing on **5% sheep blood agar**.
Note: A tube test is performed with **2%** sodium deoxycholate.
- Gently drop liquid over the colony, without dislodging colony from agar.
- Incubate plate at **35°C** for **30 minutes**.
- Examine for Lysis of colony.

Expected Results:

Positive: colony disintegrates; an imprint of the lysed colony may remain within the zone. (like A in the **figure 4**)

Negative: intact colonies. (Like B in the **figure 4**)

Quality Control:

Positive: *Streptococcus pneumoniae*.

Negative: *Enterococcus faecalis*.

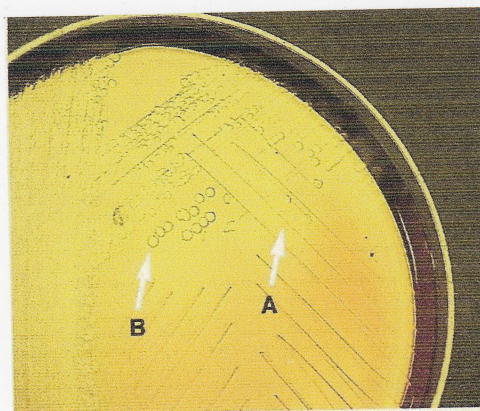


Figure (4)

V- Citrate Utilization

Short review:

Citrate utilization is the "C" portion of the IMViC test, which is used in enteric bacteria characterization; citrate is an organic molecule that utilized by bacteria capable to produce the enzyme citrase. *Klebsiella pneumoniae* produce this enzyme but *Escherichia coli* not produce it.

Principle:

This test is used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Bacteria that can grow on this medium turn the bromthymol blue indicator from green to blue.

Method:

- Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is **18 to 24 hours old**.

Note: there is **no need to stab into the butt of the tube**. Do not inoculate from a broth culture, because the inoculums will be too heavy.

- Incubate at **35°C to 37°C** for up to **24-48 hours (longer incubation "up to 7 days" may be required)**.
- Observe for development of **blue** color, denoting alkalization.

Expected Results:

Positive: Growth on the medium, with or without a change in the color of the indicator. The color change of the indicator is due to acid or alkaline production by the test organism as it grows on the medium. Growth usually results in the bromthymol blue indicator, turning from green to blue.

Negative: Absence of growth.

Quality Control:

Positive: *Klebsiella pneumoniae*.

Negative: *Escherichia coli*.

Notes to be observed:

- 1- Compare results to uninoculated control for color changes.
- 2- Look for any growth as well as you look for any color changes, due to that only citrase producing bacteria will grow onto this agar media.

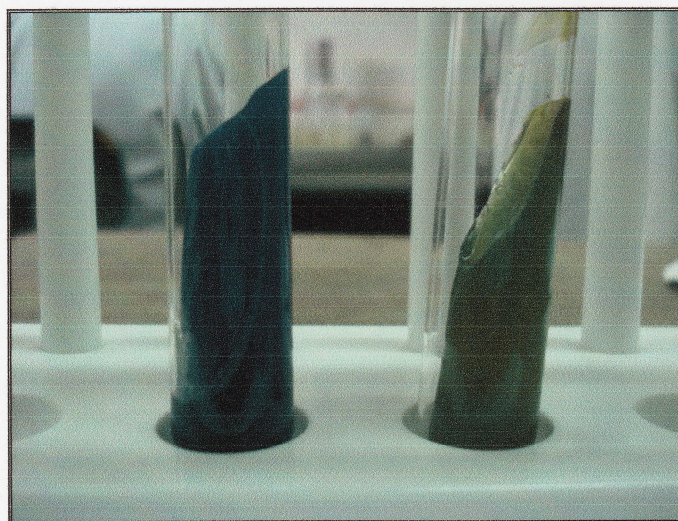


Figure (5): Shows citrate utilization positive test result with blue color regarding *Klebsiella* spp. (left), negative result with green color regarding *E.coli* (right).

VI- Growth at 42°C

Principle:

This test is used to determine the **ability of an organism to grow at 42°C.**

Method:

- Inoculate two tubes of trypticase Soya agar (TSA) with light inoculums by lightly touching a needle to the top of a single **18 to 24 hour**-old colony and streaking the slant.
- Immediately incubate one tube at **35°C** and one at **42°C.**
- Record the presence of growth on each slant after **18 to 24 hours.**

Expected Results:

Positive: Good growth at both **35°C** and **42°C.**

Negative: No growth at **42°C**, but good growth at **35°C.**

Quality Control:

Positive: *Pseudomonas aeruginosa*.

Negative: *Pseudomonas fluorescens*.

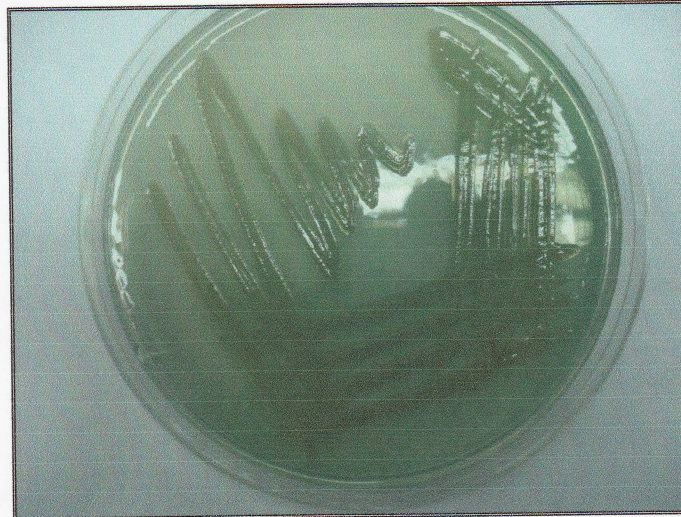


Figure (6): Shows growth ability of *Pseudomonas aeruginosa* at 42 °C in nutrient agar plate.

VII- Coagulase Test

Short review:

This test is used to differentiate *Staphylococcus aureus* (positive) from Coagulase-negative staphylococci (negative). *S. aureus* produced two forms of Coagulase: bound and free. Bound Coagulase or "clumping factor" is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the **cells to clump** when a bacterial suspension is mixed with plasma.

Principle:

The presence of bound Coagulase correlates well with free Coagulase, an extracellular protein enzyme that causes the formation of a clot when *S. aureus* colonies are incubated with plasma. The clotting mechanism involves activation of a plasma Coagulase- reacting factor (CRF), which is modified or derived thrombin molecules, to form a Coagulase-CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

There are two methods for Coagulase test to be carried out:

- 1- Slide method.(slide Coagulase test for free clumping factor only)
- 2- Tube method.(tube Coagulase test for free and bound clumping factor)

Tube Method:

- Emulsify several colonies in **0.5 ml.** of rabbit plasma (with EDTA) to give a milky suspension.
- Incubate a tube at **35°C** for **4 hours**.
- Check for clot formation.

Note: tests can be positive at **4 hours** and then revert to negative **after 24 hours**.

- If negative at **4 hours**, incubate at room temperature overnight and check again for clot formation.

Expected Results:

Positive: Clot of any size.

Negative: No clot.

Quality Control:

Positive: *Staphylococcus aureus*.

Negative: *Staphylococcus epidermidis*.

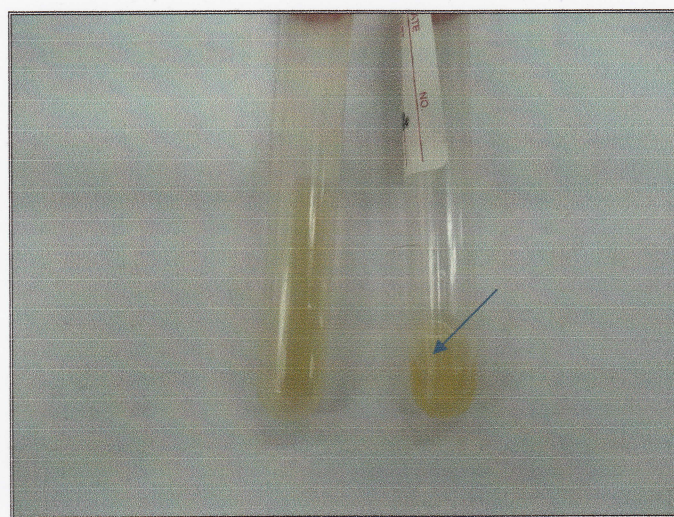


Figure (7): Shows Coagulase positive test result with clot in bottom regarding bacteria producing the enzyme Coagulase the citrated plasma has been coagulated (right), negative result without clot regarding bacteria not producing Coagulase enzyme the contents are fluid (left).

VIII- Motility Testing

Principle:

These tests are used to **determine if an organism is motile**. An organism must possess flagella to be motile.

Method:

The test is carried out by two methods:

A-Hanging drop

- Using a young (**6- to 24-hour**) actively growing **25°C** Broth culture, place 1 drop of the culture in the center of a **22 x 22-mm cover slip**.
- Place a small drop of immersion oil on each corner of the cover slip.
- Invert the cover slip over the concavity of a depression slide.
- Examine with the high dry (**X 40**) objective.

B- Semi –solid agar deep

- Touch a straight needle to a colony of a **young (18-to 24- hour)** culture growing on agar medium.
- Stab once to a depth of **only 1/3 to 1/2 inch** in the middle of the tube.
- Incubate at **35° to 37° C** and examine daily for up to **7 days**.

Expected Results:

A. Hanging drop

Positive; in true motility, the organisms change position with respect to respect to each other, often darting across the field.

Negative; In Brownian movement, the organism may appear quite active, but they remain in the same relative position to other organisms or debris in the field.

B. Semi-solid agar deep

Positive; motile organisms will spread out into the medium from the site of inoculation.

Negative; Non motile organisms remain at the site of inoculation.

Quality Control:

Positive; *Escherichia coli*

Negative; *Klebsiella pneumoniae*

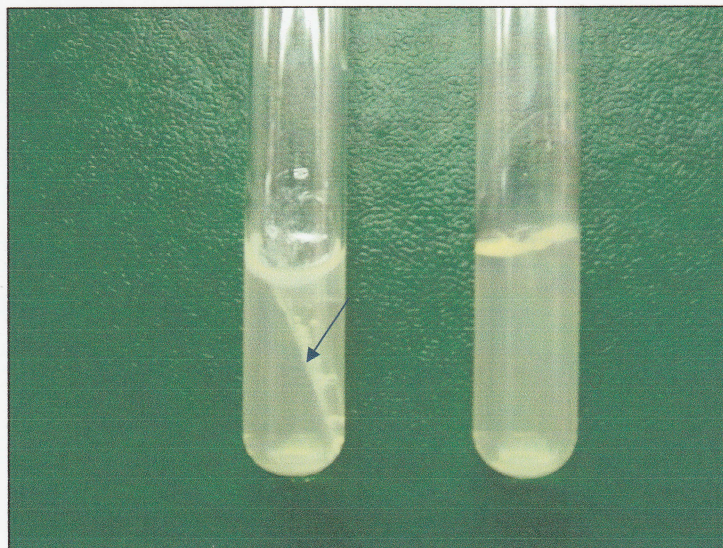


Figure (8): Shows motility test using semi-solid mannitol agar slant consisting of beef extract, peptones, and 0.5% agar which permit the bacterial cells motion, a positive test (left) regarding *E.coli*, and negative test result regarding *Klebsiella pneumoniae* (right).

IX- Optochin Test

Principle:

This test is used to determine **the effect of optochin (ethylhydrocupreine hydrochloride)** on an organism. Optochin lyses pneumococci (positive test), but alpha-streptococci are resistant (negative).

Method:

- Using an inoculating loop, streak two or three suspect colonies of a pure culture onto half of a **5%** sheep blood agar plate.
- Using heated forceps, place an optochin disk in the upper third of the streaked area. Gently tap the disk to ensure an adequate contact with the agar surface.
- Incubate plate for **18 to 24** at **35° C** in **5% CO₂**. No grow a round the disc
- Measure zone of inhibition in millimeters, including diameter of disk

Expected Results:

Positive; Zone of inhibition is 14mm or greater in diameter, with 6mm disk.

Negative; No zone of inhibition

Equivocal: any zone of inhibition less than 14mm is questionable for pneumococci; the strain is identified as pneumococci only if it is bile-soluble.

Quality Control:

Positive; *streptococcus pneumoniae*.

Negative; *streptococcus mitis*.

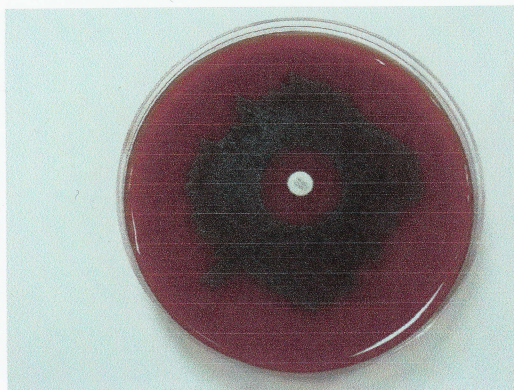


Figure (9): Shows Optochin test, showing zone of inhibition > 14mm.
(*Streptococcus pneumoniae*).

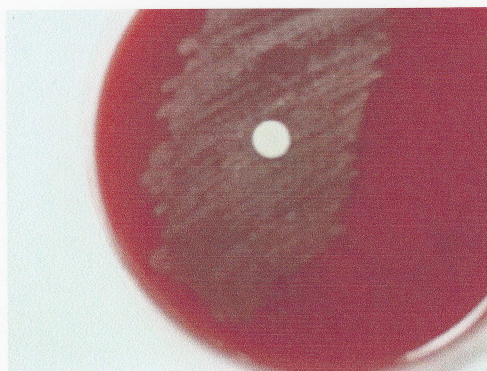


Figure (10): showing growing up to the disk (Alpha-hemolytic Streptococci).

X- Kligler's iron agar

Principle:

Can be used to direct the initial identification of gram-negative bacilli; particularly members of the Enterobacteriaceae, in KIA some organisms have the ability to ferment both carbohydrates, others ferment only glucose; still others are capable of fermenting neither lactose nor glucose., carbohydrates fermentation may occur either with or without gas production ($\text{CO}_2 + \text{H}_2$). Fermentation occurs both aerobically (on the slant) and an aerobically (in the butt).

There are three basic fermentation patterns observed on KIA medium:

- 1) glucose fermentation only.(Alkaline/Acidic)
- 2) The fermentation of both glucose and lactose.(Acidic/Acidic)
- 3) The failure to ferment glucose and lactose.(Alkaline/Alkaline)

Can detect three primary characteristics of a bacterium; the ability to produce gas from the fermentation of sugars, the production of large amounts of hydrogen sulfide gas (as visualized by the formation of a black iron-containing precipitate)

KIA contains a limiting amount of glucose and a tenfold greater lactose concentration.

Method:

A small amount of growth from a pure colony is picked onto a straight wire and inoculated to these media by streaking the surface of the slant and stabbing the butt of the tube all the way to the bottom. Only the tops of colonies growing on selective agar should be touched, since inhibited flora may be present and viable .For the same reason, the needle or loop should not be cooled in the agar of any selective medium. Gas formation is usually visualized as bubbles and cracks in the medium, caused by the pressure of the gas formed in the agar. Therefore the inoculating wire must be stabbed down the center of the agar butt of the tube; careless inoculation may allow the wire to form a channel in the agar along the inside glass wall of the tube through which the newly formed gas may escape, preventing its detection. The presence of oxygen in the atmosphere is necessary for the proper reaction to occur on the slant; therefore caps must be very loose if screw-capped tubes are used.

Enterobacteriaceae and other glucose fermenters first begin to metabolize glucose, as glucose utilizing is present constitutively and the bacteria can gain the most energy from using the simplest sugar. All other sugars must be converted to glucose before they enter the Embden-Meyerhof pathway. Glucose utilization occurs both aerobically on the slant where oxygen is available as a terminal electron acceptor and in the butt where conditions are anaerobic. Once a glucose fermenting bacterium has reduced all of the available glucose to pyruvate, it will further metabolize pyruvate via the aerobic Kerbs cycle (on the slant) to produce acid endproducts. The acid in the medium causes the PH indicator, phenol red, to

assume a yellow color. Thus, after 6 h. of incubation, both the slant and butt of a KIA that has been inoculated with a glucose fermenter will appear yellow. If the organism cannot ferment glucose, the butt will remain red (indicating no change in PH) or become alkaline (may be indicated by a red color slightly deeper than that of the original medium) demonstrating that the organism is not a member of the Enterobacteriaceae.

After depletion of the limited glucose, an organism that is able to do so will begin to utilize lactose or sucrose. Since there is 10 times as much lactose (and sucrose in KIA) as glucose in the agar, the organism will have enough substrate to continue making acid end products. The slant and butt of the KIA will remain yellow after 18-24 h. incubation. This reaction is called acid over acid (A/A) and the organism is identified as a lactose fermenter. The production of gas will cause the medium break up or to be pushed up the tube, so that a gas-producing lactose fermenter will give an A/A plus gas reaction.

If the organism being tested cannot use the lactose in the medium, it must produce energy in a less efficient way by using the protein and amino acids in the medium as a nutrient sources.

Protein metabolism occurs primarily on the surface of the slant where oxygen is plentiful. The end products of peptone breakdown (such as ammonia) are alkaline and cause the phenol red indicator to revert back to its original red color. After 18-24 h incubation of a non lactose fermenter, the KIA will thus show a red slant; the butt remains yellow due to the early anaerobic glucose metabolism. This reaction is called alkaline over acid (Alk/A or K/A).

Glucose non fermenters may also produce alkaline products from peptone utilization on the slant. Such reactions will be alkaline over alkaline (Alk/Alk or K/K) or alkaline over no change (Alk/NC).

Expected Results:

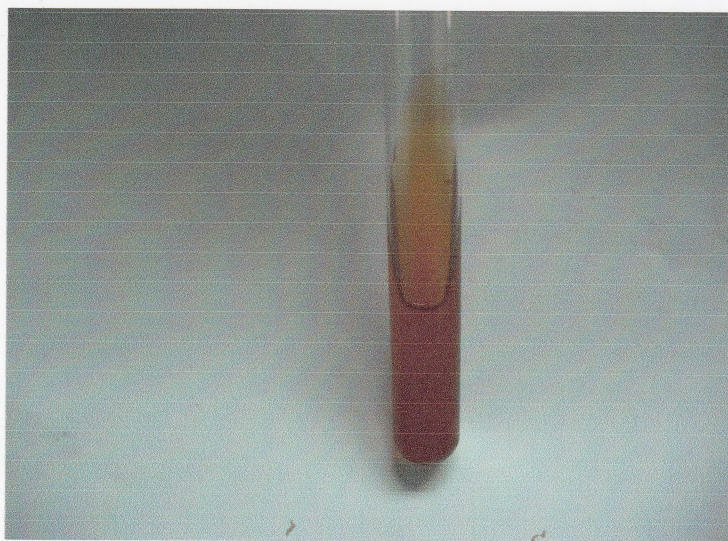


Figure (11): Shows an alkaline slant/no change in the butt (K/NC)

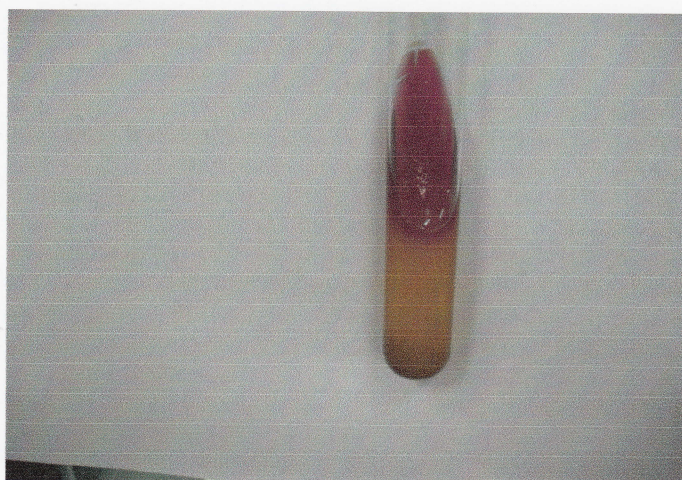


Figure (12): Shows an alkaline slant/acidic butt (K/A)

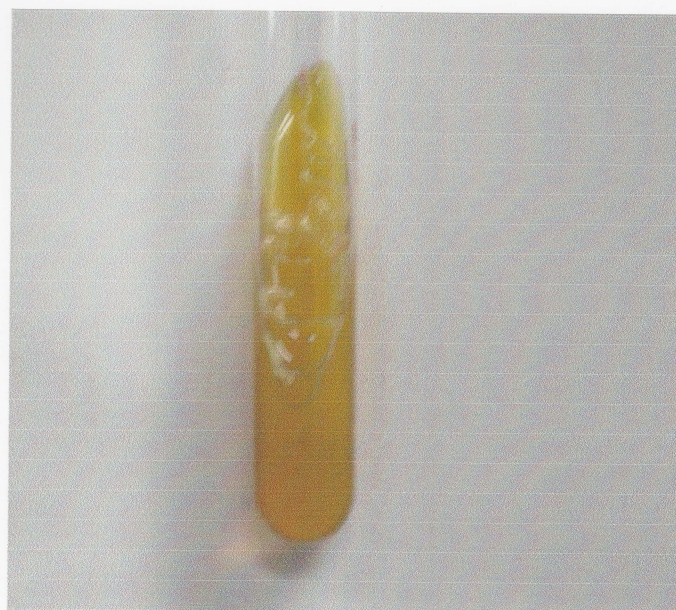


Figure (13): Shows an acidic slant/acidic butt (A/A)

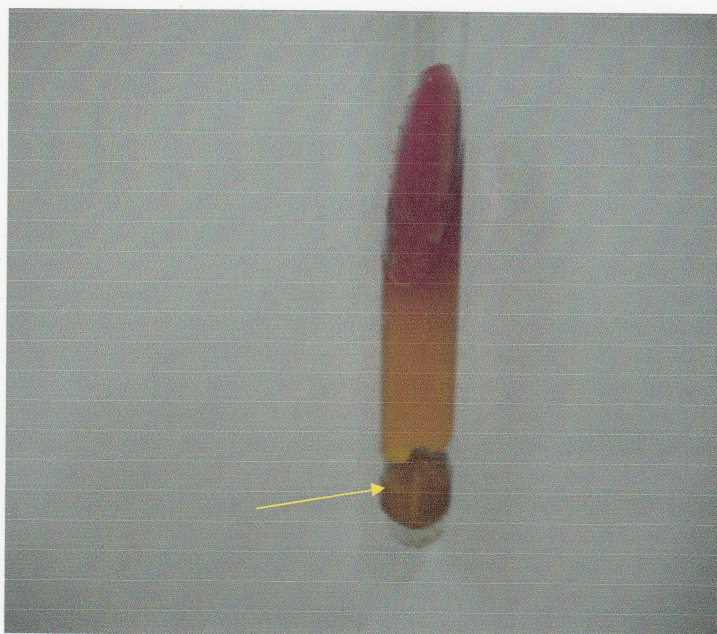


Figure (14): Black precipitate in the butt indicates production of ferrous sulfide and gas bubbles in the tube indicate the production of CO_2 or H_2S .



Figure (15): Shows an alkaline slant and acidic butt with hydrogen sulfide (H₂S) production with gas indicated via agar crackles e.g., *Salmonella typhimurium*

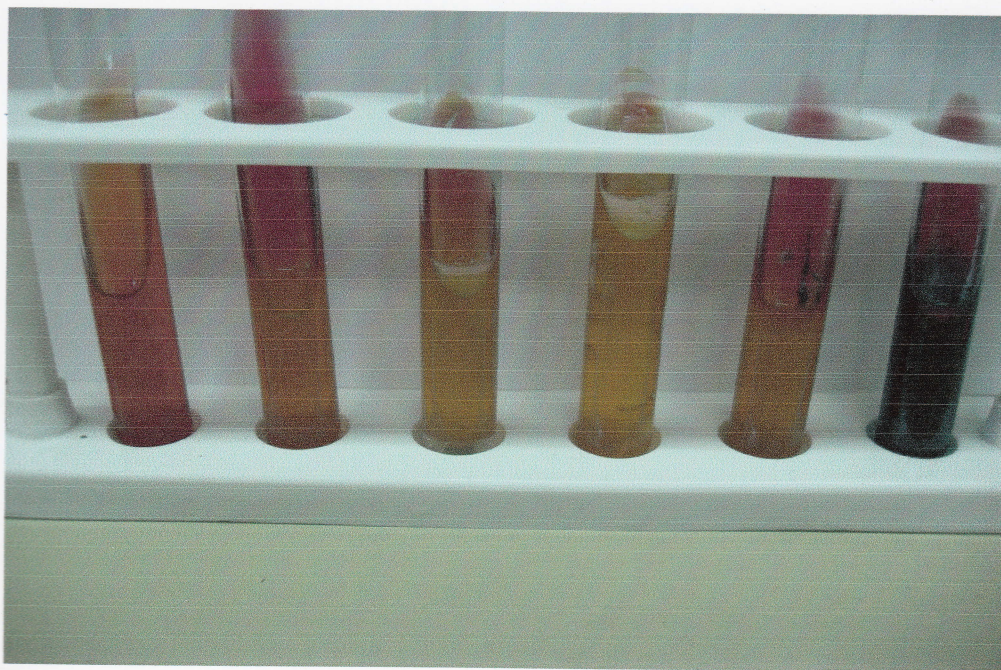


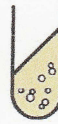




Figure (16): Shows different KIA slant reactions; (from left-right) a controlled blank, A/A without gas, A/A with gas (revealed as a crack), AK/A, AK/A with H₂S plus gas, AK/AK with H₂S plus gas production.

Alk/A gas	Alk/A no gas	A/A gas	Alk/A H ₂ S	A/A H ₂ S	Possible species
					
+	+	+	-	-	<i>Escherichia coli</i> <i>Hafnia alvei</i>
+	+	-	-	-	<i>Morganella morganii</i> <i>Providencia alcalifaciens</i> <i>P. rettgeri</i> <i>P. stuartii</i> <i>Serratia sp.</i>
+	-	+	-	-	<i>Enterobacter aerogenes</i> <i>E. cloacae</i>
+	-	+	+	+	<i>Citrobacter sp.</i>
+	+	-	+	-	<i>Salmonella sp.</i>
-	+	-	-	-	<i>Shigella sp.</i>
-	+	+	-	-	<i>Yersinia sp.</i>
-	-	+	-	-	<i>Klebsiella sp.</i>
-	-	-	+	+	<i>Proteus mirabilis</i> <i>P. vulgaris</i>
-	-	-	+	-	<i>Edwardsiella tarda</i>

*Some species show slight gas.

Chart (1): showing basic reactions of Enterobacteriaceae on Kligler's iron agar (KIA).

Urea Hydrolysis (Christensen's Method)

Principle:

This test is used to **determine the ability of an organism to produce the enzyme ureas, which hydrolyzes urea.** Hydrolysis of urea produced **ammonia** and **CO₂**. The formation of ammonia alkaline the medium and the PH shift is detected by the color change of **phenol red** from **light orange** at PH **6.8** to **magenta** at PH **8.1**.

Method:

- Streak the surface of a urea agar slant with a portion of a well isolated colony or inoculate slant **1 to 2 drops** from an overnight brain-heart infusion broth culture.
- Leave the cap on loosely and incubate the tube at **35°C** in ambient air for **18 to 24 hours to 7 days**.

Expected Results:

Positive: change in color of slant from **light orange** to **magenta**.

Negative: no color change (agar slant and butt remain **light orange**)

Quality Control:

Positive: *Proteus vulgaris*.

Negative: *Escherichia coli*

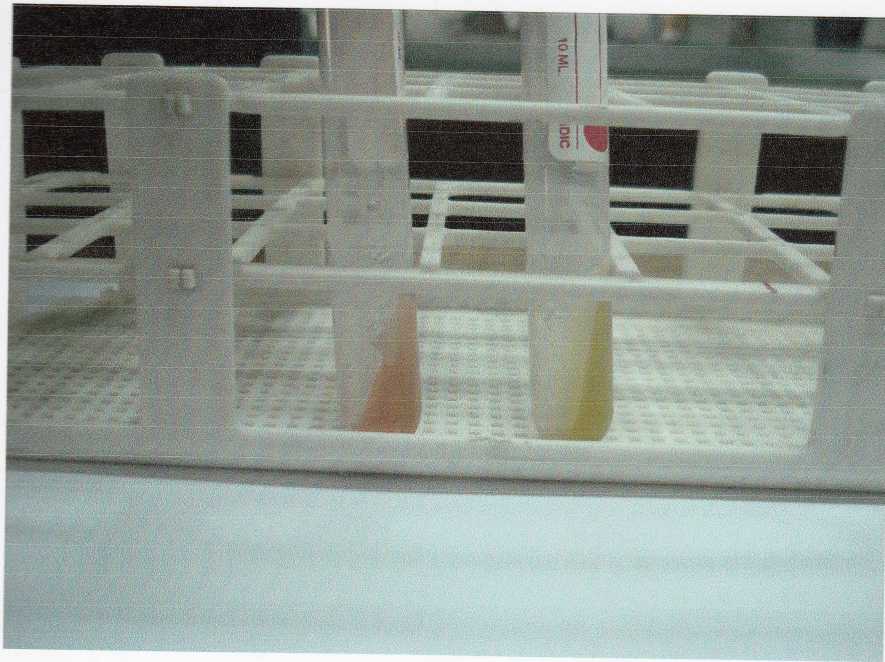


Figure (17): Shows urea utilization positive test by regarding *Proteus vulgaris*, light orange [left], a negative test result regarding *E.coli*, yellow [right].

Table (1): shows different laboratory specimens manipulations methods.

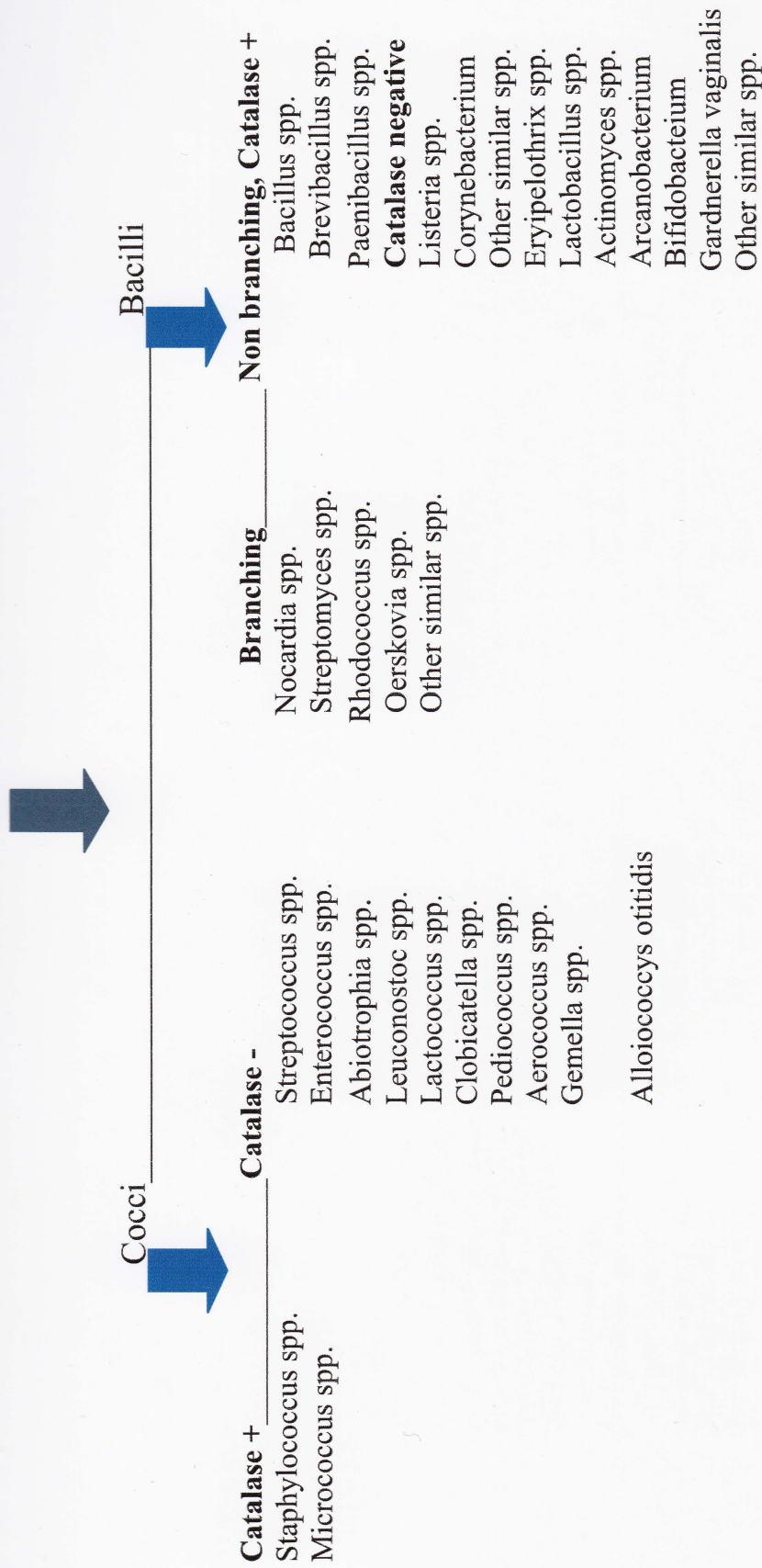
Specimen	Primary plating media	Direct examination	Expected microorganism	Diagnosis
Urine	B.A, M.A	Deposit of urine	Enterobacteriaceae(e.g , <i>E.coli</i> , <i>Klebsiella</i> spp.) <i>Pseudomonas</i> and other non fermenter microorganisms, <i>Streptococcus</i> (<i>Enterococci</i>) <i>Staphylococcus saprophyticus</i> , other <i>Staphylococci</i>	1-Fewer than $10^{4.4}$ cells per ml. (absence of UTI) ما عدا اذا : (1) سحب الادرار من المثانة مباشرة (2) وجدت اعراض المرض عند المرأة (3) وجدت الخلايا القلبية في الادرار $2-10^{4.4}-10^{4.5}$ cells per ml. بدون اعراض يعاد الزرع وان وجدت يعتبر UTI 3-More than $10^{4.5}$ cells per ml. يعتبر التهاب بسبب نوع واحد أو نوعين من البكتريا وأن كانت أكثر من نوعين يعتبر تلوث ويعاد النموذج.
Blood	Brain heart infusion then sub culture to Ch.A, B.A(aerobic + CO_2), M.A, Sab.,if need B.A anaerobic	Gram stain	<i>Salmonella typhi</i> & non typhi, <i>Brucella</i> spp., <i>Streptococcus viridans</i> S. aureus, coagulase negative staphylococci, <i>Streptococcus pyogenes</i> <i>Streptococcus pneumoniae</i> , <i>Neisseria meningitidis</i> , <i>Haemophilus influenzae</i> , <i>P.aeruginosa</i> , Other Enterobacteriaceae, <i>Candida albicans</i>	حصول تغيير في الـ blood bottle واي نمو يعتبر اصابة بعد التأكد من ظروف السحب والزرع , قد لا يحصل أي تغيير عند الإصابة ببعض أنواع البكتريا مثل <i>Haemophilus influenzae</i> <i>Streptococcus pneumoniae</i> ملاحظة: كل نموذج يجب أن يشخص لأن الدم يجب ان يكون Sterile

CSF	B.A(aerobic + CO ₂ & anaerobic), M.A, Ch.A, Sab.	Gram stain Z.N.stain	<i>Haemophilus influenzae</i> , <i>Neisseria meningitidis</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus pneumoniae</i> , <i>Listeria monocytogenes</i> , <i>Enterobacteriaceae</i> , <i>Mycobacterium tuberculosis</i> , <i>Cryptococcus neoformans</i>	تؤخذ بنظر الاعتبار وان كانت مستعمرة واحدة
Stool	Hekton enteric agar, G.N. broth , XLD, M.A, TCBS, , Alkaline peptone water	Direct examination (saline & Iodine)	<i>S. typhi</i> & <i>S. paratyphi</i> & other <i>salmonella</i> spp. <i>Shigella</i> , <i>Vibrio cholerae</i> , <i>Yersinia enterocolitica</i> <i>Aeromonas</i> & <i>Campylobacter</i> spp.	*over 50 pus cells/h.p.f in shigellosis. Less 20 pus cells/h.p.f in invasive <i>E.coli</i> , <i>Salmonella</i> spp 2-5 pus cells/h.p.f in ETEC, EPEC, Cholera & viral diarrhea.
Upper respiratory tract	B.A with CO ₂ , Ch.A, Tellurite blood agar, Thayer -Martin medium, M.A, Sab.	Gram stain	<i>S.pneumoniae</i> (ear & sinus), <i>S.pyogenes</i> (throat), <i>S.aureus</i> (ear & sinus), <i>Pseudomonas</i> spp., <i>Neisseria meningitidis</i> , <i>Moraxella catarrhalis</i> (ear & sinus), <i>Haemophilus influenzae</i> (ear & sinus), <i>Corynebacterium diphtheriae</i> (throat & nose), <i>Candida albicans</i> (oropharynx)	أحيانا N.F ان كانت تعتبر Pathogenic عند الأشخاص المثبتين مناعيا.

L.R.T	B.A with CO ₂ , Ch.A, M.A, Lowenstein- jensen medium(if required for T.B.	Gram stain,Z.N stain	<i>Moraxella catarrhalis</i> , <i>S.pneumoniae</i> , <i>S.aureus</i> , <i>K.pneumoniae</i> , <i>H.influenzae</i> , Enterobacteriaceae, <i>Mycobacterium tuberculosis</i> , <i>Pseudomonas</i> spp. <i>Candida albicans</i> ,	Heavy&Pure كانت أحياناً N.F.ان تعتبر Pathogenic عند الأشخاص المصابين مناعياً."
Pus& exudates	B.A(aerobic +CO ₂ & anaerobic), M.A, Thioglycolate broth	Gram stain, Z.N stain	Enterobacteriaceae, <i>Pseudomonas</i> & other non-ferment, <i>S.aureus</i> , <i>S.pyogenes</i> <i>Mycobacterium tuberculosis</i> , <i>M. ulcerans</i> , Other <i>Mycobacterium</i> spp., <i>Bacillus anthracis</i> , <i>Bacteroides</i> & other strict anaerobic, <i>C.perfringenes</i> , <i>Pasteurella multocida</i> .	
Fluid(deposit -e after centrifugatio -n if clear fluid)	B.A(aerobic +CO ₂ & anaerobic), M.A, Ch.A , Sab.	Direct exam Gram stain Z.N stain	According to the type of fluid	Any fluid must be sterile

N.B. BA = Blood agar, MA= MacConkey agar, CA=Chocolate agar, Sab. =Sabouraud dextrose agar, Z.N.=Ziehl Neelsen stain,G.N.broth =gram negative broth, TCBS=thiosulphate citrate bile salts sucrose agar, XLD= xylose lysine desoxycholate agar.

Gram Positive Aerobic Bacteria



Scheme (2): aerobic and facultative anaerobic gram-positive bacteria

Gram-Negative Aerobic Bacteria



Cocci

Neisseria gonorrhoeae
Neisseria meningitidis
Other Neisseria spp.
Moraxella catarrhalis

Bacilli/Coccobacilli



Mac.A growth

Oxidase-negative

Enterobacteriaceae
Acinetobacter spp.
Stenotrophomonas
Maltophilia
Oxidase-positive
Pseudomonas spp.
Burkholderia spp.
Achromobacter spp.
Rhizobium spp.
Ochrobactrum spp.
Chryseobacterium spp.
Sphingobacterium spp.
Alcaligenes spp.
Bordetellae
Comamonas spp.

Mac.A no growth

Oxidase-variable

Haemophilus spp.
Oxidase-positive
Sphingomonas
paucimobilis
Moraxella catarrhalis
Neisseria elongate
Eikenella corrodens
Weeksella virosa
Pasteurella spp.
Suttonella indologenes
Mannheimia haemolytica
Actinobacillus spp.
Kingella spp.
Cardiobacterium spp.
Capnocytophaga spp.

Growth requires special media

Bartonella spp.
Afipia spp.
Campylobacter spp.
Arcobacter spp.
Helicobacter spp.
Legionella spp.
Brucella spp.
Bordetella pertussis
Bordetella parapertussis
Francisella tularensis
Streptobacillus
moniliformis
Spirillum minus

Scheme (3): gram negative aerobic bacteria

Catalase- Positive, Gram-positive cocci

Family: (Cocciceae)

1 - Genus Staphylococcus:

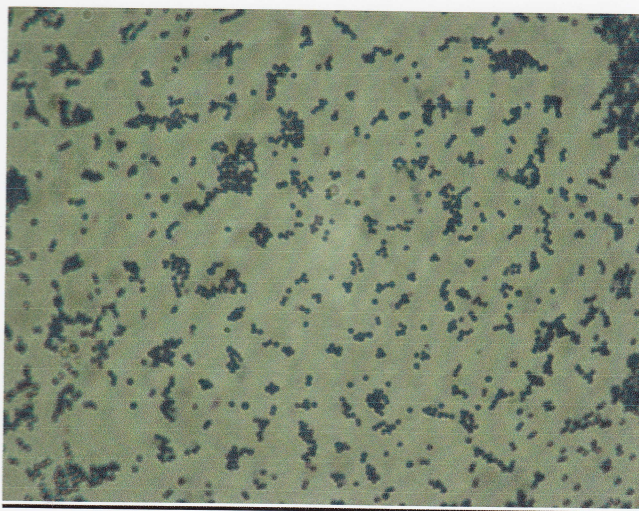


Figure (18): A Gram stained film of 24 hours culture revealed *Staphylococcus aureus*. Notice the uniform result (all cells are purple i.e., Gram positive reaction). In addition cocci found in clusters due to cell division, which occurs in more than one plane (100X).

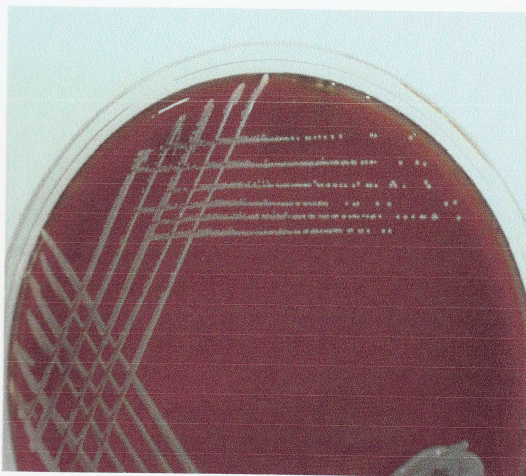


Figure (19): A streak plate of *Staphylococcus aureus* onto blood agar plate (BA). These organism colonies are smooth, convex, shiny, and moderate, with yellowish tint, sometimes surrounded by a narrow zone of hemolysis.

Table (2): The properties of important *Staphylococci* species.

Bacterial species	pigment	Coagulase	novobiocin	Mannitol to acid
<i>S. aureus</i>	+	+	S	+
<i>S. epidermidis</i>	-	-	S	-
<i>S. saprophyticus</i>	V	-	R	+

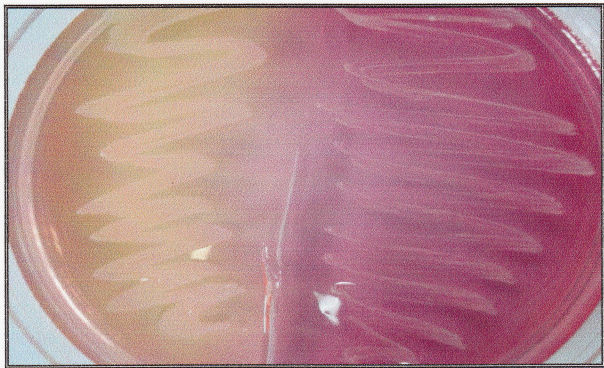


Figure (20): Mannitol salt agar inoculated with *S. aureus* confirmed by Coagulase test (at left) and *S. epidermidis* (at right). Both grew on this medium, but only *S. aureus* ferment Mannitol with acid production, which change agar color to yellow.

Catalase- Negative, Gram –Positive Cocci

2- Genus *Streptococcus and Enterococcus:*

They are Gram-positive chains forming cocci, facultative anaerobes, and non-spore forming.

The Enterococci involved four species, *E.faecalis*, *E.faecium*, *E.durans*, and *E. avium*. They are responsible for humans UTIs, infective endocarditis, and septicemia.

They produce acid from arabinose, mannitol, raffinose, pyruvate, and sorbitol. Ammonia produced from arginine, also they hydrolyzed esculin in bile-esculin agar plate, and finally they produce an enzyme called pyrrolidonyl peptidase which gave positive PYRase test reaction.

Table (3): Colonial appearance and characteristics on 5% sheep blood agar.

Organism	Appearance
Group A beta-hemolytic streptococci ^a	Grayish white, transparent to translucent, matte or glossy; large zone of beta hemolysis
Group B beta-hemolytic streptococci ^b	Larger than group A streptococci; translucent to opaque; flat, glossy; narrow zone of beta hemolysis; some strains nonhemolytic
Group C beta-hemolytic streptococci ^c	Grayish white, glistening; wide zone of beta hemolysis
Group F beta-hemolytic streptococci ^d	Grayish white, small, matte; narrow to wide zone of beta hemolysis
Group G beta-hemolytic streptococci ^e	Grayish white, matte; wide zone of beta hemolysis
<i>S. pneumoniae</i>	Small, gray, glistening; colonies tend to dip down in the center and resemble a doughnut (umbilicated) as they age; if organism has a polysaccharide capsule, colony may be mucoid; alpha-hemolytic
Viridans streptococci ^f	Minute to small, gray, domed, smooth or matte; alpha-hemolytic or nonhemolytic
<i>Abiotrophia</i> spp. and <i>Granulicatella</i> spp. ^g	Resemble viridans streptococci
<i>Enterococcus</i> spp.	Small, cream or white, smooth, entire; alpha-, beta-, or non-hemolytic

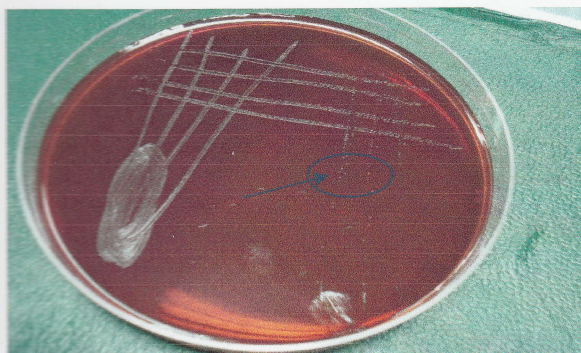


Figure (21): Shows MacConkey's agar without crystal violet plate streaked by *Streptococcus faecalis* isolated from 44 years old Iraqi male with discharging abdominal fistula post colonic carcinoma resection. Observed the entire, convex, opaque, small, Catalase-negative colonies.

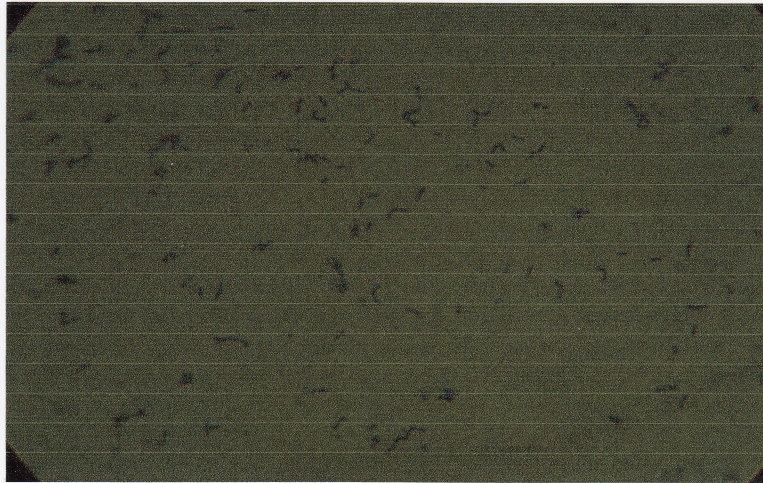


Figure (22): A Gram stained film of *Streptococcus faecalis* (Enterococcus) from abdominal fistula discharging in 44 years old Iraqi male patient colostomy was carried on him previously. Notice the organism short chains (100X).



Figure (23): Chains of cocci (*Streptococci*) in *Streptococcus pyogenes* isolated from a throat swab. Here the cell division occurs in one plane; hence the cells remain together in one line for a distance (100X).

Streptococcus pyogenes:

Gram-positive spherical cocci, capsulated, non-motile, non-spore forming, facultative anaerobic. Colonies are small, semi-transparent, low convex, clear, wide zones of haemolysis surround colonies on horse sheep blood agar.

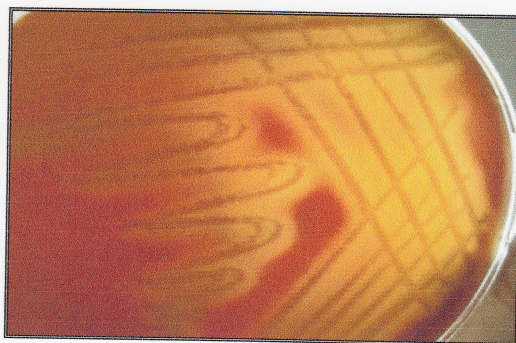


Figure (24): *Streptococcus pyogenes* isolated from 4 years old Iraqi male patient with impetigo. Notice the beta-hemolysis, indicated by a clearing around the colonies onto BA plate.

Bacitracin sensitivity; *Streptococcus pyogenes* is more sensitive to bacitracin than other streptococcus, a disc containing 0.04 units of bacitracin is placed on the well of the primary culture plate, *Streptococcus pyogenes* should show a large inhibition zone (e.g. >14 mm diameter) and most other streptococci show little or no inhibition. (Positive: any zone of inhibition around the disk, Negative: No zone of inhibition around the disc)

Table (4): Differentiation of β -haemolytic streptococci

Species	<i>S. pyogenes</i>	<i>S. agalactiae</i>	<i>E. faecalis</i> var. <i>zymogenes</i> ^a
Lancefield group	A	B	D
Haemolysis	β	β^b	β
Zone around the differential bacitracin disc	+	0 ^c	0 ^c
Bile-aesculin agar (growth & blackening)	0	0	+
Reverse CAMP test	0	+	0
Co-trimoxazole ^e susceptibility	0	0	0
PYR test ^f	+	0	+

E. faecalis var. *zymogenes* produces β -haemolysis only on horse blood agar.

5% are non-hemolytic.

5% are positive.

10% are positive.

PYR:pyrrolidonyl- β -naphthylamide.

Streptococcus pneumoniae:

Gram-positive cocci occurring in pairs (diplococci) or, usually short, chains. They are ovoid or lanceolate in shape, with their distal ends narrowed. They are non-motile and non-spore-forming, the polysaccharide capsule is the key to the organism's virulence.

Colonies on blood agar are small, smooth and transparent. Low convex while tiny, they become flattened or depressed centrally, showing the "draughtsman form". Partial clearing of blood and a greenish discoloration (alpha-haemolysis) is produced underneath and in a narrow zone around the colonies. Generally narrower zones of alpha-haemolysis help to identify the *pneumococci*. Growth may be better anaerobic than aerobically and the haemolysis may then resemble the beta hemolytic type.

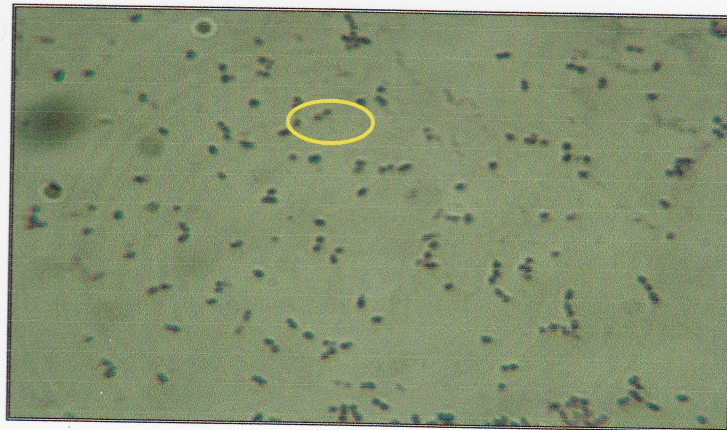


Figure (25): A Gram-stained film shows Gram-positive diplococci [lancet shape] isolated from a sputum specimen streaked onto a chocolate agar plate, suspected to be *S.pneumoniae*; confirm our result via the optochin diagnostic disc test result. It found singly, or in pairs, or in short chains (100X).



Figure (26): Revealed growth of *Streptococcus pneumoniae* onto blood agar plate. Notice the alpha-hemolysis (green color) around the small, tiny, convex with plateau, opaque, the colonies are without β -hemolysis unlike *S pyogenes*.

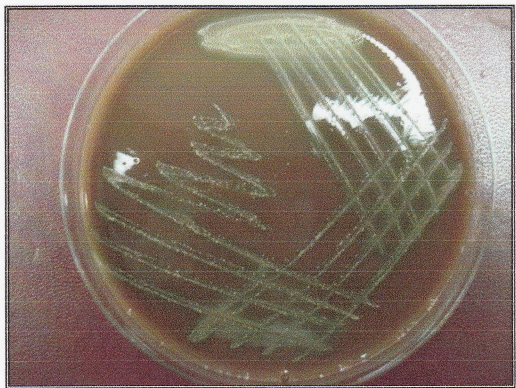


Figure (27): Growth of *Streptococcus pneumoniae* isolated from 24 years old female Iraqi leukemia patient. Notice the alpha-hemolysis (green color) around the small, tiny, convex with plateau, opaque, glistening colonies onto chocolate agar plate.

Table (5): Shows different characters belonging *S.pneumoniae* and *S.viridans*.

Character	<i>Pnemococcus</i>	<i>S.viridans</i>
Morphology	ovoid or lanceolate diplococci;some short chains	Short or long chains of rounded cocci usually absent
Capsule	Present	Usually absent
Colonies	Become flattened or draughtsman	Convex
Effect on blood agar	Narrow zones of α -hemolytic	Wide or narrow zone of α-hemolytic
Optochin sensitivity	Sensitive	Resistant
Bile solubility	(+)	(-)

Enterococci: the enterococci have the group D,they are part of the normal enteric flora. They are usually nonhemolytic, but occasionally

α -hemolytic. Enterococci are PYR-positive, they grow in the presence of bile and hydrolyze esculin. (Bile esculin-positive).

They grow in 6.5% NaCl. they grow well at between 10°C and 45°C whereas streptococci generally grow a much narrower temperature range. Resistant to penicillin G than the streptococci.

There are at least 12 species of enterococci. *Enterococcus faecalis* is the most common and causes 85-90% of enterococcal infections, while *Enterococcus faecium* causes 5-10%.

In patients, the most common sites of infection are the urinary tract, wounds, biliary tract, and blood.

Enterococci may cause meningitis and bacteremia in neonates. In adults, the enterococci can cause endocarditis.

Enterococcus faecalis; Occurs in ovoid pairs or short chains, and is non-motile and non-capsulate. It's grow readily on ordinary nutrient media and on MacConkey's agar without crystal violet, it forms small magnates colored colonies, its usually non-hemolytic, but sometimes alpha or beta -hemolytic.

Enterococcus faecium:

Found as normal flora in the human gastrointestinal tract, can able to grow in 6.5% NaCl.

Non -Branching, Catalase-Positive, Aerobic, Gram-Positive Bacilli

Genus: *Listeria monocytogenes*:

They are gram positive, short on slightly curved rods with rounded ends, some have rudimentary branching. cells are arranged singly, in palisades of parallel cells, or in pairs that remain connected after cell division to form V or L shapes groups of these morphologies seen together resemble and are often referred to as Chinese letters gram positive rod that may *Listeria monocytogenes* is a short occur singly or in short chins resembling streptococci.

Listeria monocytogenes can be identified by observation of motility by direct wet mount. the organism exhibits characteristic end over-end tumbling motility when incubated in nutrient broth at room temperature for 1 to 2 hours, and can be seen by an umbrella-shaped pattern that develops after overnight incubation at room temperature of culture stabbed into a tube of semi solid agar, flagella and show tumbling motility at 20-25°C. not at 35-37°C; occasional non-motile strains have been isolated

Listeria monocytogenes ferments glucose and is voges-proskauer positive and esculin-positive. the isolation of a small gram-positive, Catalase-positive rod with a narrow zone of beta-hemolysis isolated from blood or cerebrospinal fluid(CSF) should be used as strong presumptive evidence for listeriosis.

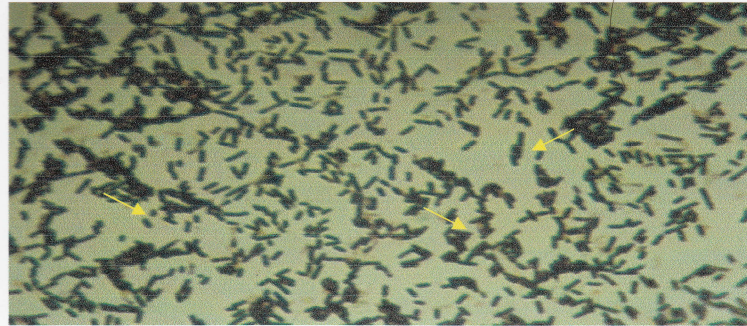


Figure (28): Revealed the gram film of *Listeria monocytogenes*; notice the short gram-positive rods or coccobacilli.

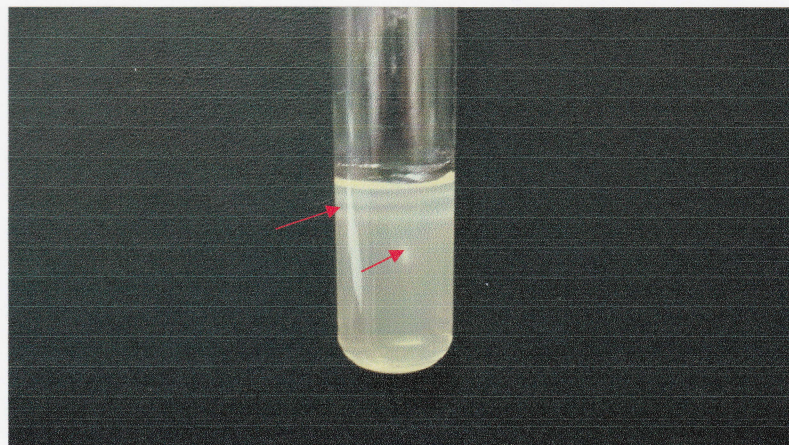


Figure (29): Shows inoculated semisolid Mannitol agar with Catalase-positive *L.monocytogenes*; notice the characterized umbrella motility mode.

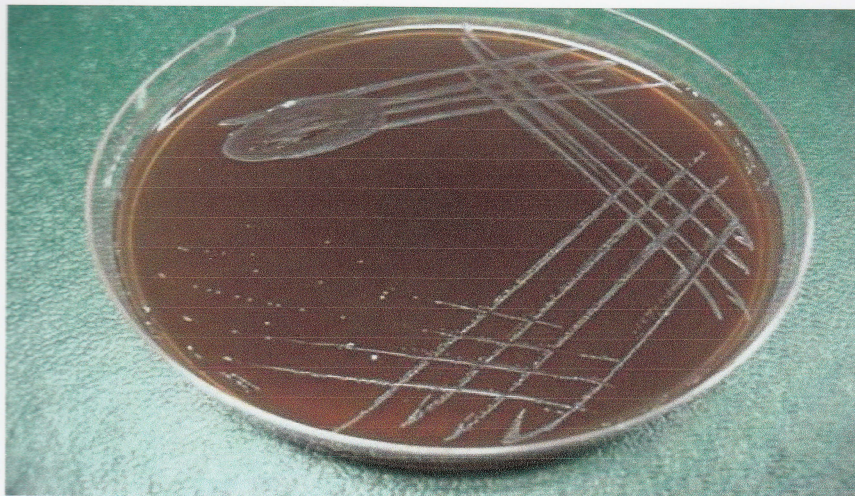


Figure (30) shows small, white, smooth, translucent, moist, beta-hemolytic *Listeria monocytogenes* colonies onto blood agar plate, isolated from acute meningitis case in young Iraqi immunocompromised female leukemia patient.

Genus:

Corynebacterium diphtheriae:

Irregularly staining, pleomorphic gram-positive rods produce colonies ranging from small, gray, and translucent to medium, white, and opaque. *Corynebacterium diphtheria* biotype mitis may be beta-hemolytic; selective and differential media for *Corynebacterium diphtheria* should be used if diphtheria is suspected. the two media commonly used for this purpose are cystine-tellurite blood agar and modified tins dale's agar. in addition, Loeffler medium, containing serum and egg. Stimulates the growth of *Corynebacterium diphtheria* and the production of metachromatic granules within the cells.

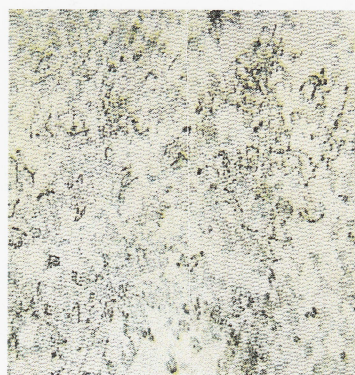
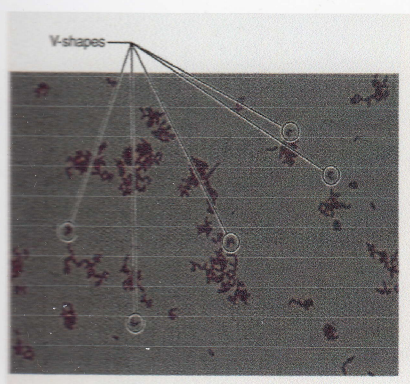


Figure (31) V-shape in *Corynebacterium diphtheriae* after binary fission the two cells remain together at one end to yield the angular arrangement seen here.

Gram Negative Bacilli and Coccobacilli (MacConkey –Positive, Oxidase –negative)

Family;

Enterobacteriaceae

They are a large, heterogeneous group of gram negative rods, grow aerobically and facultative anaerobically on simple media; they are Oxidase negative and Catalase positive; they ferment glucose and other carbohydrates.

Table (6): Shows the colonial appearance of the family *Enterobacteriaceae* onto different culture media.

Organism	Medium	Appearance
<i>Citrobacter</i> spp.	Mac	Late lactose fermenter; therefore, NLF after 24 hours; LF after 48 hours; colonies are light pink after 48 hours
	HE	Colorless
	XLD	Red, yellow, or colorless colonies, with or without black centers (H ₂ S)
<i>Edwardsiella</i> spp.	Mac	NLF
	HE	Colorless
	XLD	Red, yellow, or colorless colonies, with or without black centers (H ₂ S)
<i>Enterobacter</i> spp.	Mac	LF; may be mucoid
	HE	Yellow
	XLD	Yellow
<i>Escherichia coli</i>	Mac	LF; flat, dry, pink colonies with a surrounding darker pink area of precipitated bile salts
	HE	Yellow
	XLD	Yellow
<i>Hafnia alvei</i>	Mac	NLF
	HE	Colorless
	XLD	Red or yellow
<i>Klebsiella</i> spp.	Mac	LF; mucoid
	HE	Yellow
	XLD	Yellow
<i>Morganella</i> spp.	Mac	NLF
	HE	Colorless
	XLD	Red or colorless
<i>Proteus</i> spp.	Mac	NLF; may swarm depending on the amount of agar in the medium; characteristic foul smell
	HE	Colorless
	XLD	Yellow or colorless, with or without black centers
<i>Providencia</i> spp.	Mac	NLF
	HE	Colorless
	XLD	Yellow or colorless
<i>Salmonella</i> spp.	Mac	NLF
	HE	Green
	XLD	Red with black center
<i>Serratia</i> spp.	Mac	Late LF; <i>S. marcescens</i> may be red pigmented, especially if plate is left at 25° C (Figure 22-3)
	HE	Colorless
	XLD	Yellow or colorless
<i>Shigella</i> spp.	Mac	NLF; <i>S. sonnei</i> produces flat colonies with jagged edges
	HE	Green
	XLD	Colorless
<i>Yersinia</i> spp.	Mac	NLF; may be colorless to peach
	HE	Salmon
	XLD	Yellow or colorless

HE, Hekto enteric agar

LF, Lactose fermenter (pink colony)

Mac, MacConkey agar

NLF, Non lactose fermenter, Colorless colony

XLD, Xylose –Lysine-Deoxycholate agar.

Gram Negative Bacilli and Coccobacilli (MacConkey –Positive, Oxidase –negative)

Family;

Enterobacteriaceae

They are a large, heterogeneous group of gram negative rods, grow aerobically and facultative anaerobically on simple media; they are Oxidase negative and Catalase positive; they ferment glucose and other carbohydrates.

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	XLD	Yellow
<i>Escherichia coli</i>	Mac	LF; flat, dry, pink colonies with a surrounding darker pink area of precipitated bile salts
	HE	Yellow
	XLD	Yellow
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	XLD	Yellow
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	XLD	Red or colorless
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	XLD	Yellow or colorless
<i>Salmonella</i> spp.	Mac	NLF
	HE	Green
	XLD	Red with black center
<i>Serratia</i> spp.	Mac	Late LF; <i>S. marcescens</i> may be red pigmented, especially if plate is left at 25° C (Figure 22-3)
	HE	Colorless
	XLD	Yellow or colorless
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	HE	Green
	XLD	Colorless
<i>Yersinia</i> spp.	Mac	NLF; may be colorless to peach
	HE	Salmon
	XLD	Yellow or colorless

HE, Hekto enteric agar

LF, Lactose fermenter (pink colony)

Mac, MacConkey agar

NLF, Non lactose fermenter, Colorless colony

XLD, Xylose –Lysine-Deoxycholate agar.

Genus:

***Bacillus*:**

Large, rectangular, gram positive rods, some of which contain spores, seen as unstained, usually oval spaces, one in each stained mother cell (sporangium), *Bacillus anthracis* non motile while *Bacillus cereus* motile. Most strain indole negative, simmon citrate variable.

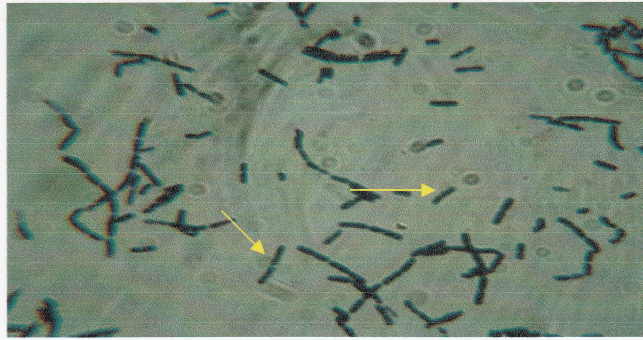


Figure (32): A 24 hour's culture of Gram-positive *Bacillus* spp., some cells undergoing binary fission and looks like Gram-positive diplococci. The bacterial shape like *E.coli* but longer, and bluish (100X).

On nutrient agar at 37° C, colonies are grayish, granular discs, 2-3 mm in diameter after 24 h, with an even margin which gives them a " Medusa head" appearance. Each is a continuous, convoluted chain of bacilli; and has a sticky, membranous consistency which makes it difficult to emulsify.



Figure (33): A streak on to blood agar plate (BA). Notice the dull off –White, non-hemolytic colonies; there is no medusa –heads outside the colonies growth just like *B-anthraxis*



Figure (34): A Gram stained film. Focused on Gram-negative rods (*E.coli*) isolated from urinary tract infection (UTI) case in young Iraqi female patient aged 24 years old (100X).

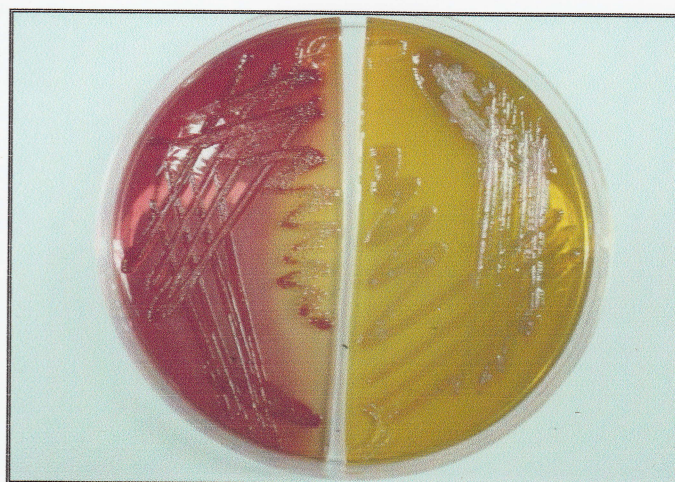


Figure (35): A MacConkey's agar plate streaked by lactose-fermenter (LF) *E.coli* (left), non-lactose fermenter (NLF) *Salmonella typhi* (right). It revealed that the LF appeared red or pinkish while the NLF appeared colorless onto this differential medium.

Genus:

Escherichia:

Most strains recovered in clinical laboratory ferment lactose and thus grow as smooth, glossy, pink colonies on MacConkey's agar. They grow as yellow colonies on xylose lysine deoxycholate agar; at least 80% of strains are motile.

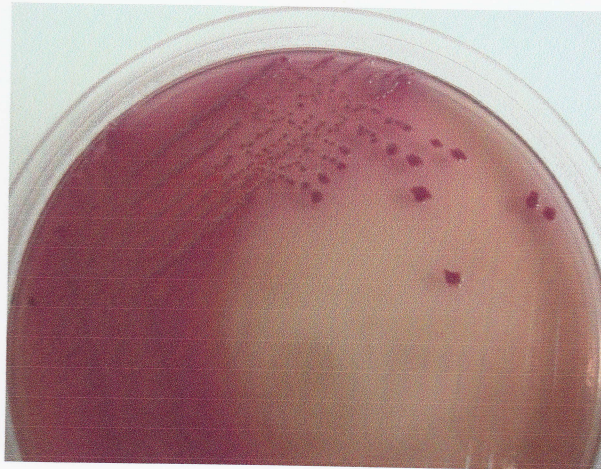


Figure (36): A MacConkey's agar plate streaked with *E.coli* aged 18-24 hours isolated from a diarrheal case (Traveler's or Tourist diarrhea); from 21 years old Iraqi female patient, observed the dry pinkish, circular, entire, convex, opaque colonies.

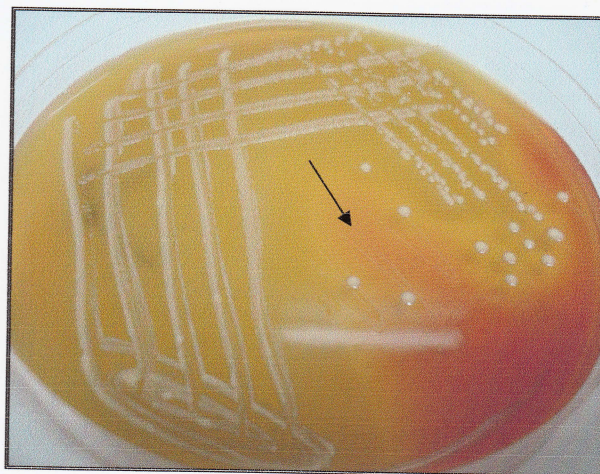


Figure (37): Xylose-lysine deoxycholate (XLD) agar plate streaked with *E.coli* isolated from adult Iraqi male complaining from acute diarrhea. Notice that the lactose-fermenter *E.coli* appeared yellowish in color.



Figure (38): Notified the biochemical reactions of *Escherichia coli*; (from left-right) Indole (+), motile, onto KIA slant AK/A and in certain circumstances A/A with gas production & no H₂S, Simmon's citrate (-), urease (-).

Genus:

Citrobacter

These bacteria typically are citrate-positive and differ from the salmonella in that they do not decarboxylate lysine. They ferment lactose very slowly if at all (late lactose fermenter).



Figure (39): shows the biochemical reaction results carried out to *Citrobacter freundii* isolated from a urine specimen (from left-right), urea negative, Simmon's citrate positive, motile, and onto KIA alkaline slant with acidic butt with H₂S, indole positive.

Genus;

Klebsiella

Lactose fermenter, are non motile, they are usually capsulate and can be recognized by their large, mucoid colonies on laboratory medium, most species hydrolyses urea but do so much less rapidly than species of *Proteus*.

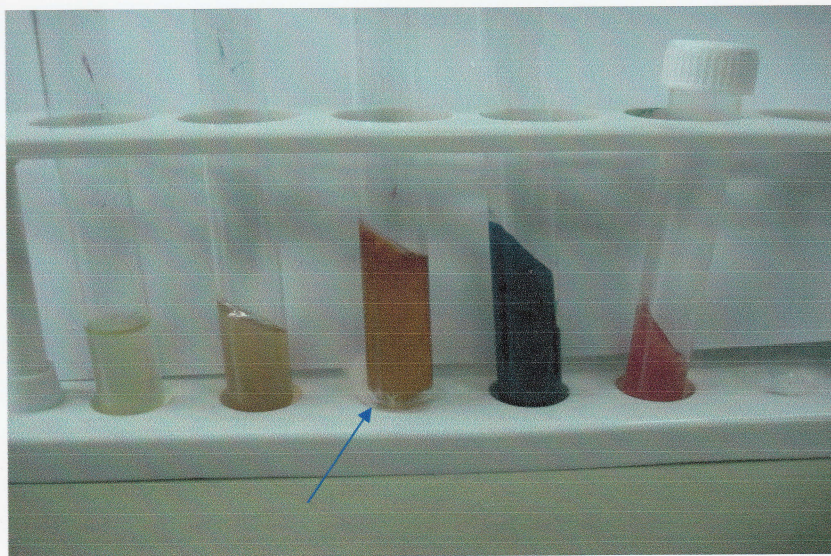


Figure (40): Revealed the biochemical reaction results of *K.pneumoniae*; (from left-right) urease (+), Simmon's citrate (+), onto KIA slant gave A/A without gas or H₂S productions, indole (-), non motile.

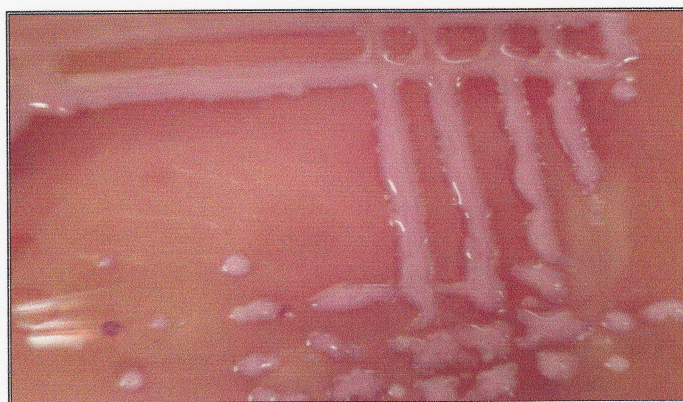


Figure (41): MacConkey's agar plate shows *Klebsiella spp.* colonies growing that isolated from a UTI case in 23 years old Iraqi female patient. Notice the sticky colonies.

Genus;

Enterobacter

Motile, urease variable, utilize citrate, indole negative, the reaction in KIA is acidic slant, and the butt is acidic also with no H₂S production.



Figure (42): Clarified the circular, opaque, convex, smooth, shiny, moderate in size, red lactose-fermenter *Enterobacter cloacae* colonies onto MacConkey's agar plate; notice the mucoid colonies which resembling *Klebsiella pneumoniae* colonies.

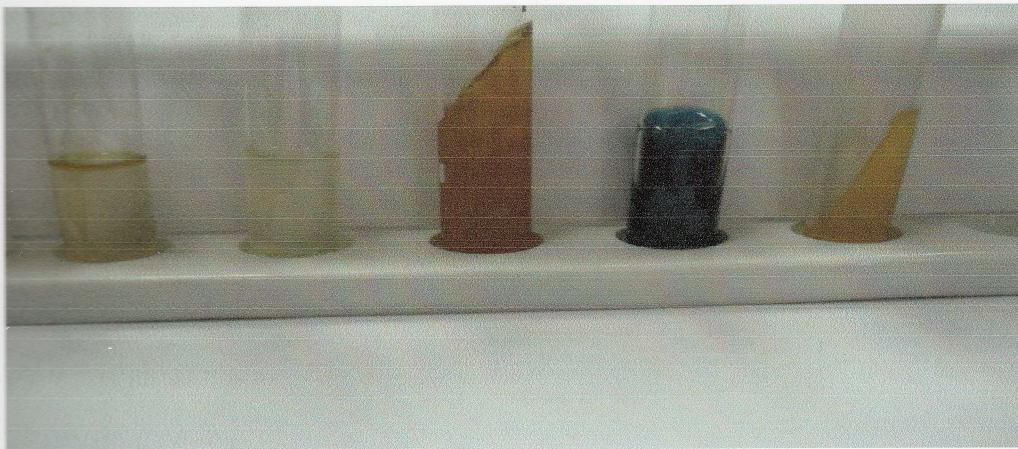


Figure (43): Shows the biochemical reactions of *Enterobacter cloacae* (from left-right) indole (-), motile, onto KIA slant gave A/A with out H₂S productions, Simmon's citrate (+), urease is variable; here is (-).

Genus;

Serratia

Usually motile; ferment glucose with acid, 99% sucrose ferment and Mannitol ferment, lactose fermentation and gas production are variable; grow on Simmons citrate medium, 15% urease positive. Pathogenic strains produce pigment.

Some species are important human pathogens, isolated from respiratory tract, wounds, blood and urine.

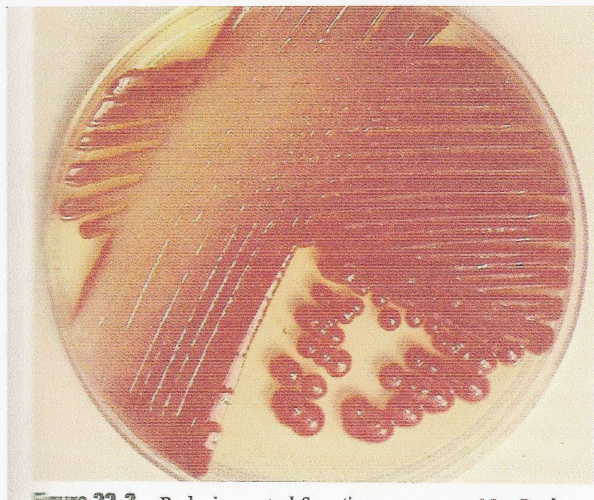


Figure (44) Clarified pinkish colonies of *Serratia* species onto macConkey's agar plate isolated from blood culture, notice the convex, opaque, circular colonies.

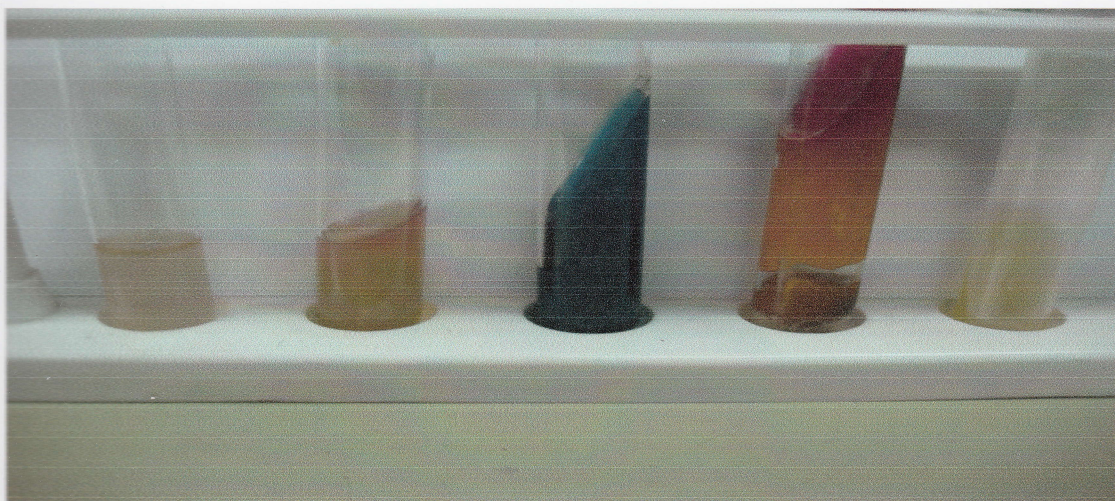


Figure (45) Shows the biochemical reactions regarding *Serratia marcescens*, notice (left-right) urea(-), Simmon citrate(+), Kligler's agar slant AK/A, motile, Indole (+).

Genus;

Proteus

Is motile, lactose non-fermenting, hydrolyze urea rapidly, swarming phenomena seen in *Proteus mirabilis*, *P. penneri* and *P. vulgaris* only.

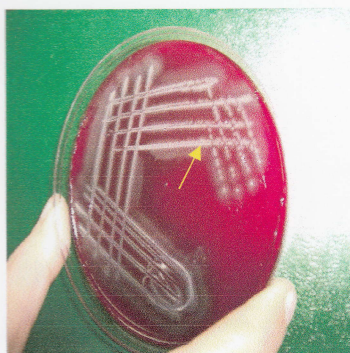


Figure (46): Revealed the growth of *Proteus mirabilis* isolated from a urine specimen; notice the migration of the organism across the blood agar surface resulting in swarming phenomenon (Expanding rings).



Figure (47): Revealed the biochemical reaction results of *Proteus mirabilis* (from left-right) indole (-) if it positive mean it's *P. vulgaris*, motile, onto KIA gave A/A with H₂S productions (most strains produce H₂S), Simmon's citrate (variables); here is (-), urease (+).

Genus;

Salmonella

Gram-negative bacilli, non capsulate, motile. After 24 h at 37° C, colonies of most strains are moderately large, gray-white, moist, circular discs with smooth convex surface and entire edge, thus resembling the colonies of many Enterobacteriaceae. Produce hydrogen sulfides.

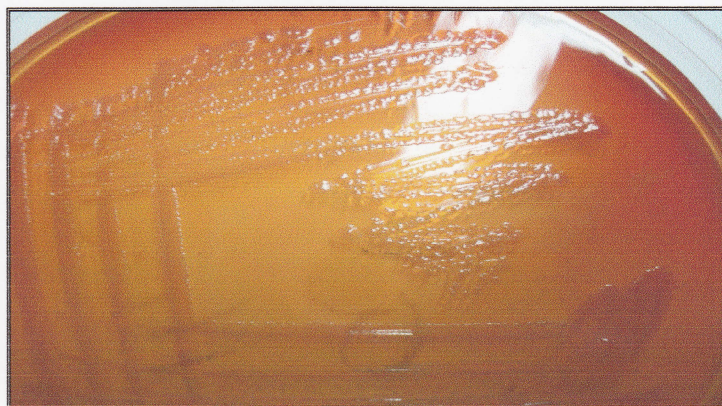


Figure (48): Shows growth of *Salmonella typhimurium* onto MacConkey's agar plate.



Figure (49): Revealed the NLF *Salmonella typhi* onto SS-agar plate isolated from young Iraqi febrile male aged 25 years old. Observed the black colonies due to H₂S production by reaction with the iron in the medium.

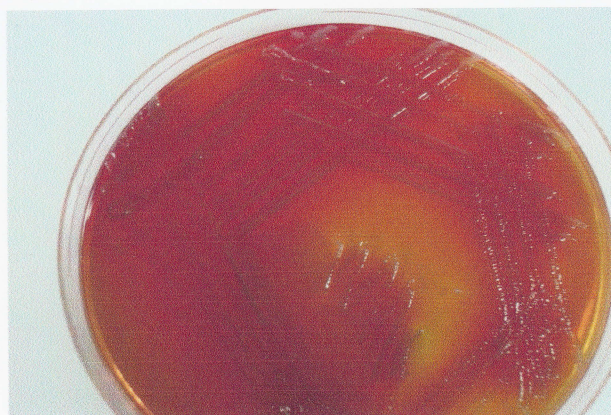


Figure (50): Shows xylose lysine deoxycholate (XLD) agar plate inoculated with *Salmonella typhimurium*. Notice that the pathogenic NLF appeared red, while the LF appeared yellowish in color.



Figure (51): shows the biochemical reaction results of *Salmonella typhi* isolated from blood culture; the result from left- right was: indole negative, motile in semisolid agar, KIA acidic slant and acidic butt with H₂S without gas, Simmon's citrate negative, and urea negative.

Genus;

Shigella

Non-sporing, non-capsulate gram-negative rods, non motile, grow on Nutrient agar and blood agar appear smooth, grayish or colorless, translucent colonies resembling those of salmonella. On MacConkey's agar; colonies are pale or colorless

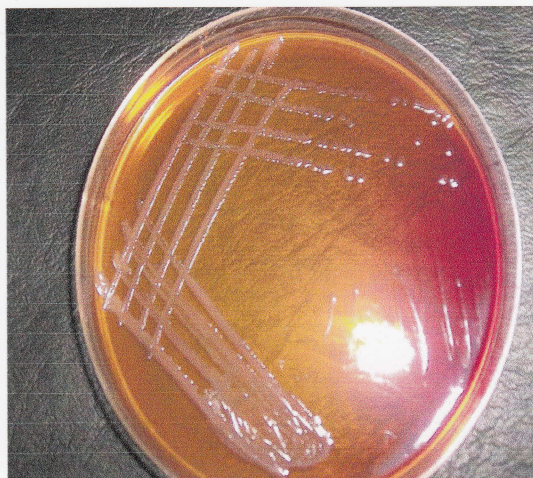


Figure (52): Revealed the colorless colonies of *Shigella* species onto MacConkey's agar plate, which resembling the *Salmonella* species colonies.

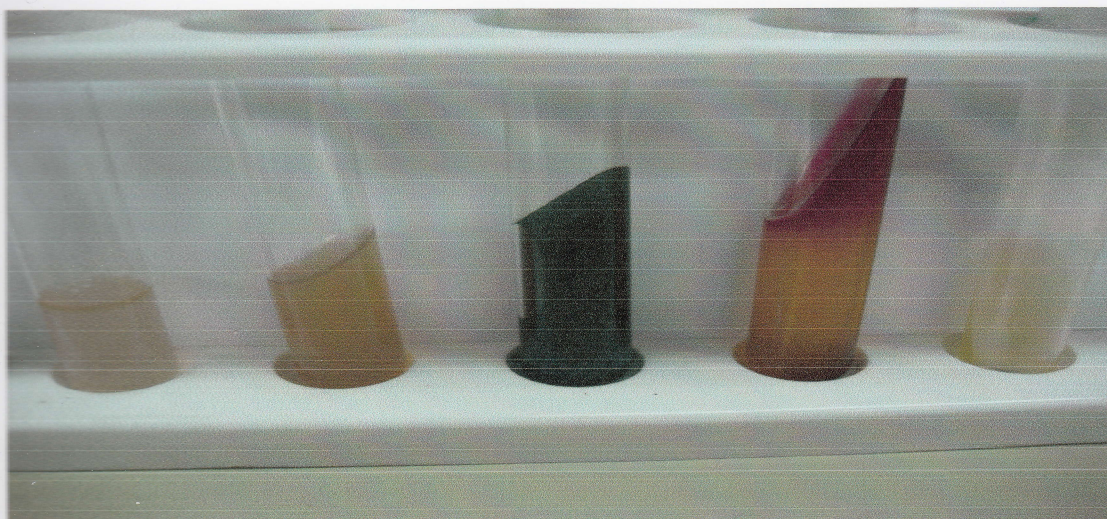


Figure (53): Shows the biochemical reaction results of *Shigella* group D identified via specific antiserum isolated from stool culture; the result from left- right was: Simmon's citrate negative, Kligler's iron agar alkaline slant and acidic butt and no H₂S, non-motile in semisolid agar slant, into indole gave negative reaction, urea negative reaction.

Genus;

Acinetobacter

Aerobic gram negative, usually coccobacillary or coccal in appearance; they resemble *Neisseria* on smears, rod-shaped forms also occur, and occasionally the bacteria appear to be gram positive, no attack glucose by oxidation or fermentation. oxidase negative, grow on MacConkey agar.

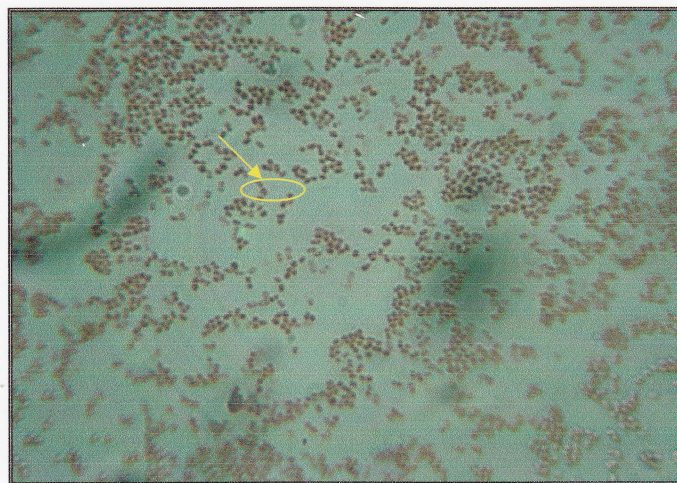
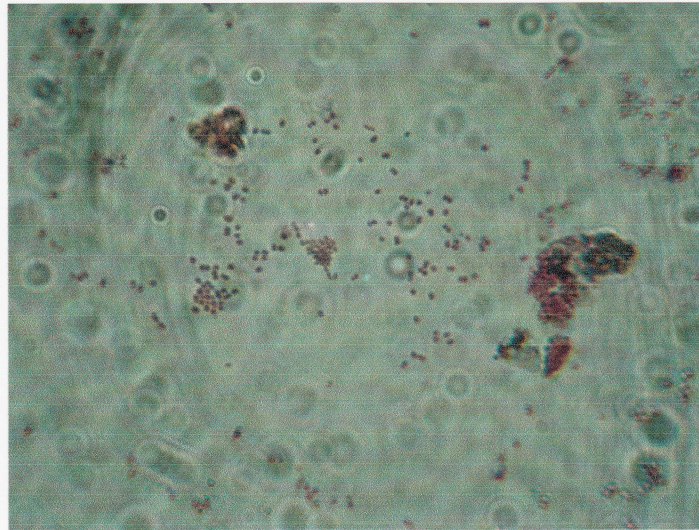


Figure (54): Gram stained film. Revealed the Gram-negative coccobacilli, *Acinetobacter baumannii* plus *M. catarrhalis* have the same morphology (100X).

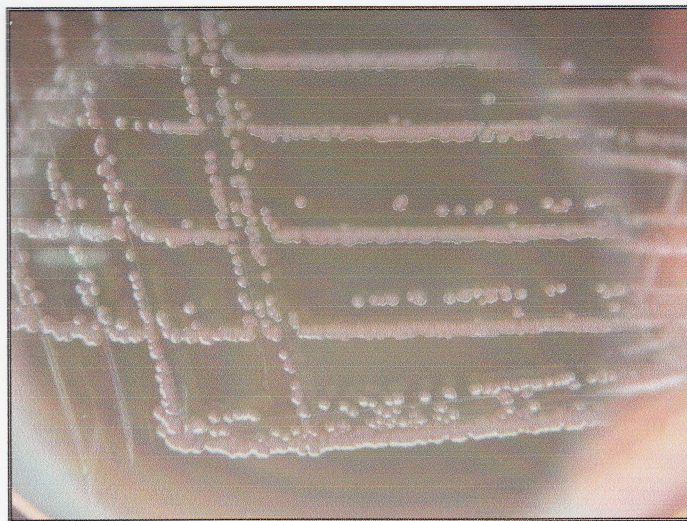


Figure (55): A streak of *Acinetobacter baumannii* onto MacConkey's agar plate. Note the pinkish hue colonies to light lavender although it's usually NLF.

Gram Negative Bacilli and Coccobacilli (MaCconkey –Positive, Oxidase –Positive)

Genus; *Pseudomonas*

Gram negative, non lactose fermenter, inert carbohydrate fermentation,; take glucose by oxidation, motile, strict aerobic; Oxidase positive and Catalase positive, grow on simple media, growth on blood agar may produce diffuse haemolysis, culture produce grape-like smell of aminoacetophenone,, the colonies are large, low convex, circular and have an irregular spreading edge rough in appearance or small, smooth, produce soluble pigment especially at room temperature, the yellow\green pigment pyoverdine (fluorescein) is also produced by some strains, giving the characteristic blue-green appearance of infected pus or cultures, other species produce other type of pigments like pyocyanin or pyomelanin and pyorubin..

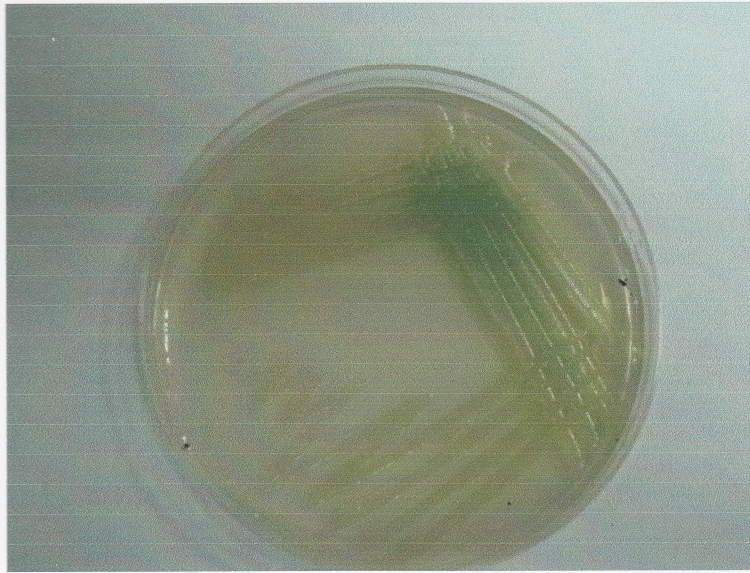


Figure (56): Revealed the growth of *Pseudomonas aeruginosa* onto a nutrient agar plate; notice the yellowish-greenish pigment (pyoverdinin; fluorescein) produced via the microbe isolated from a burn case.

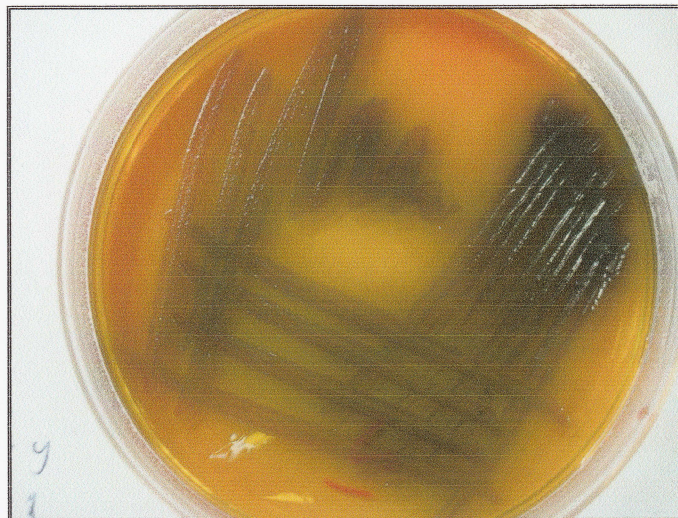


Figure (57): A streak onto MacConkey's agar plate. Revealed the growth of *Pseudomonas aeruginosa* with brown-melanin pigmentation due to the melanin pigmentation produced via the microbe in the agar.

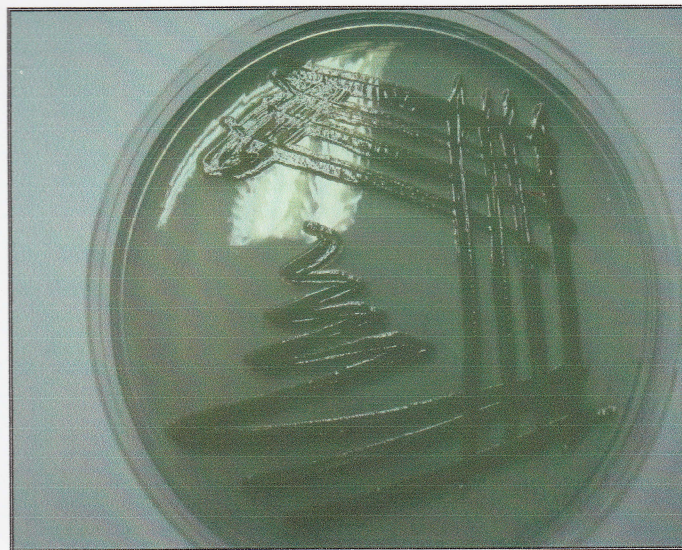


Figure (58): A streak onto Mueller-Hinton agar plate. Revealed the characteristic fruity odor and the yellowish-greenish pigmentation (pyoverdinin or fluorescence) around *Pseudomonas aeruginosa* colonies isolated from recurrent otitis media case.

Burkholderia (Pseudomonas) cepacia:

Grow as smooth, glistening red/purple colonies of approximately 2mm diameter, specific strains may show dwarf colonies appearance and a diffusible brown melanin-like pigmentation which is enhanced in medium containing 1% tyrosine.

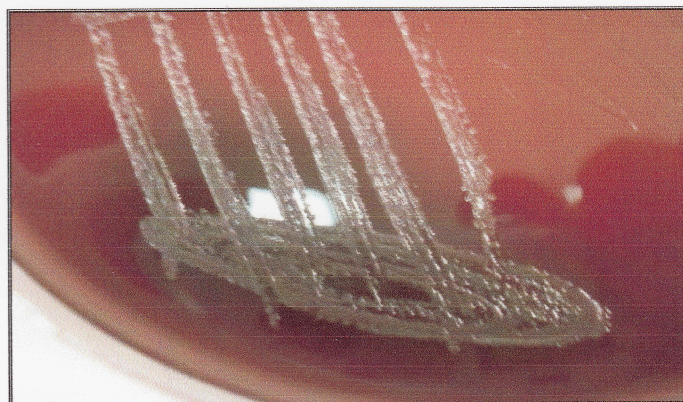


Figure (59): Shows *Pseudomonas (Burkholderia cepacia)* isolated from a sputum specimen in a child complaining from respiratory distress suspected to get cystic fibrosis. Notice the smooth non-serrated edges, opaque, gray, non-hemolytic colonies, dirtlike odor. It incubated at 35°C for 72 hours; and 14% gave negative oxidase test result.

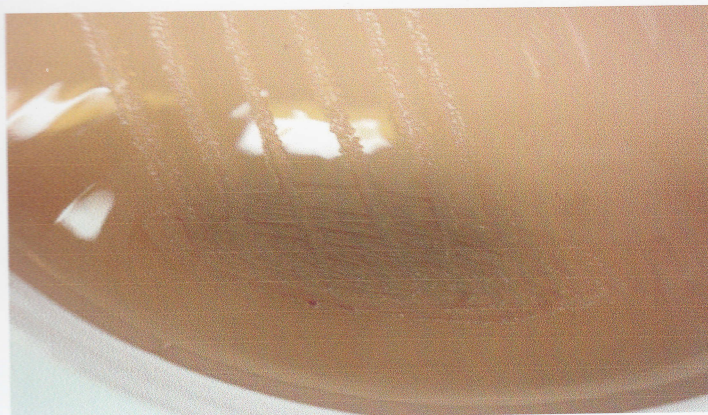


Figure (60): Shows the NLF colonies of *P.cepacia* onto MacConkey's agar plate. Observed the entire, convex colorless non-fruity odor colonies, colonies become dark pink to red due to oxidation of lactose after 4-7 days.

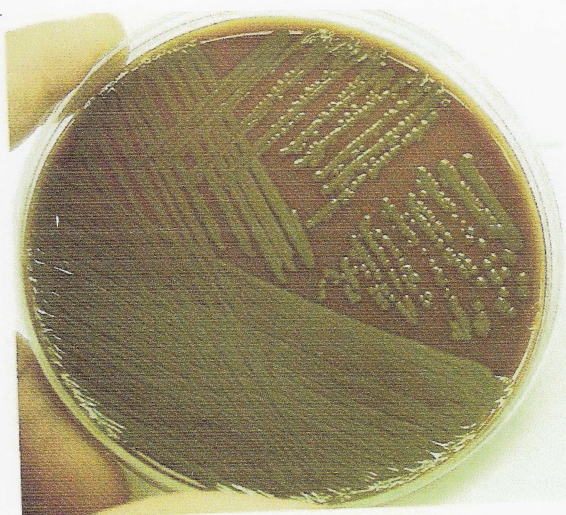


Figure (61): Shows the colonies of *P.cepacia* onto chocolate agar plate. Notice the entire, convex yellowish -green colonies.

Family; vibrioceae

Genus;

Vibrio

They are short, often curved, gram negative rods that are motile by means of a single polar, oxidase positive, mostly halophilic, but *V.cholerae* is non -halophilic, can grow in ordinary media and broth such as nutrient broth, peptone water. Most strains grow well on MacConkey's agar as non lactose ferment, giving pale colonies in 24-36h, on blood agar, zones of haemolysis are produced by hemolytic strains. On nutrient agar, colonies are glistening and translucent.

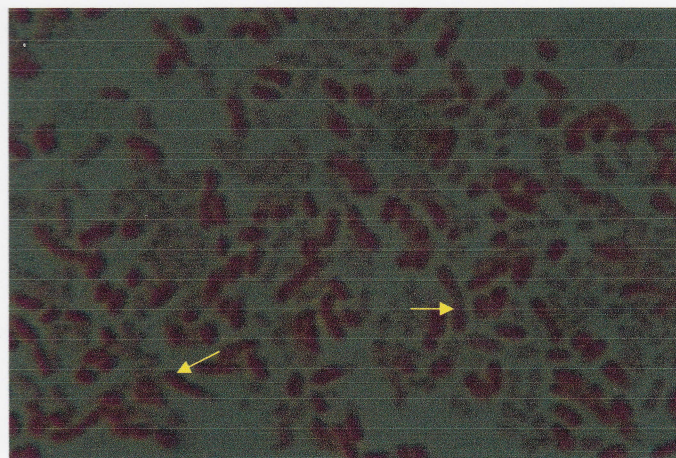


Figure (62): Clarified the gram stain slide of *V.cholerae*; notice the curved (comma shaped) gram-negative rods with an oxidase positive result from KIA.



Figure (63): Shows the biochemical reaction results of *V.cholerae* (from left-right) indole (+), motile, Simmons's citrate (+), onto KIA gave AK/A without gas without H₂S productions, urease (-).

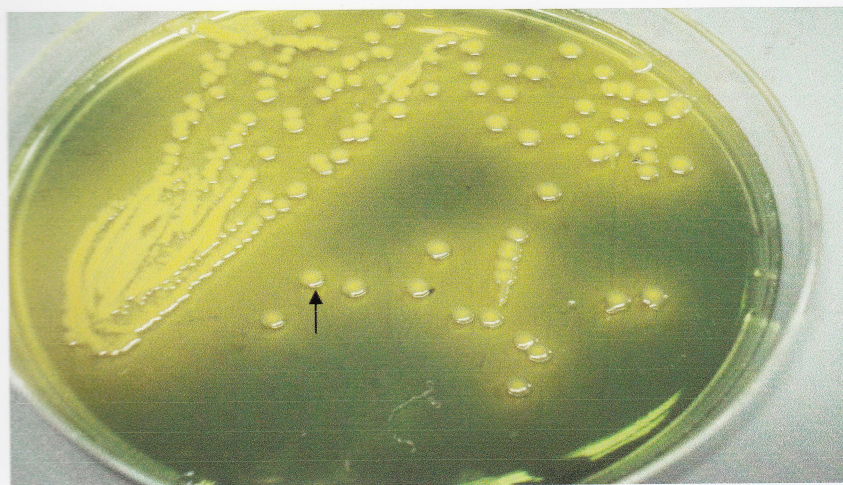


Figure (64): A sub culturing from alkaline peptone broth onto TCBS (thiosulfate citrate bile salt sucrose agar plate) yielded a yellowish, convex, entire, smooth, moderate, opaque, colonies due to *Vibrio cholerae*. Confirmed via positive oxidase test plus the biochemical test results and better by use the system of Api 20 E, Its halophilic can grow well into 6% NaCl, but not in 20%.

Genus; *Aeromonas*

In gram stain slide appear straight gram negative rods, motile, some non motile strains are found, on blood agar most strains give wide zones of beta-haemolysis, produce oxidase and catalase and ferment glucose and other substrate with production of acid and gas.

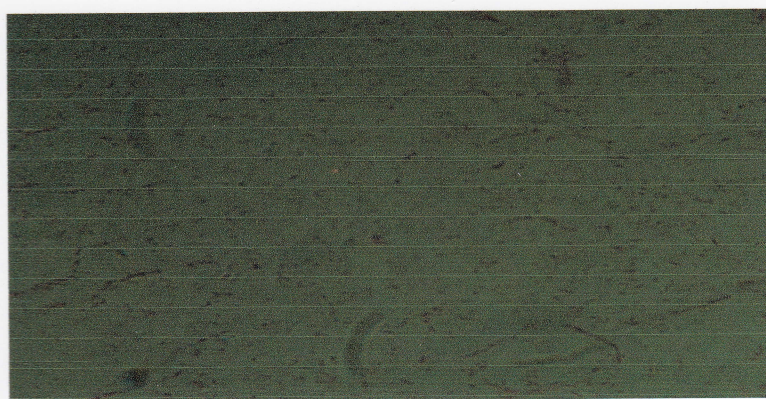


Figure (65): A Gram stained film shows Gram-negative straight rods, oxidase positive, suspected to be *Aeromonas hydrophila*, it's not a halophilic microbe like *Vibrio*.

To differentiate between *Vibrio* spp. and *Aeromonas* spp. do string test (0.5%) solution of Na-deoxycholate on slide and mix small amount of growth from the MacConkey's into the drop after 60 second *Vibrio cholerae* become mucoid.

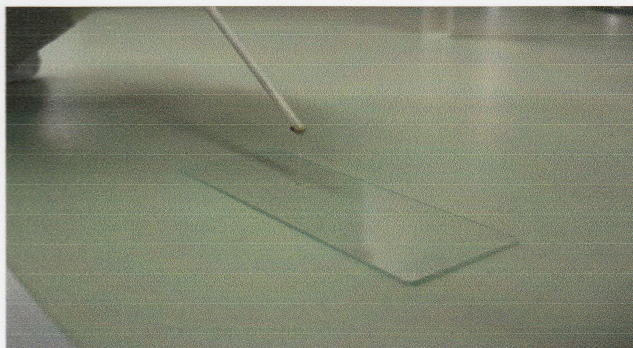


Figure (66) revealed the characteristic "String Test" belonging *V.cholerae* organism, notice the mucus thread extend from the stick to the slide surface.

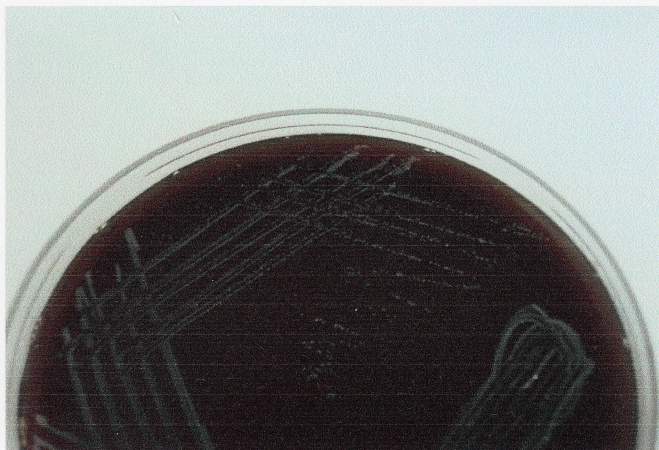


Figure (67): Oxidase positive, suspected colonies of *Aeromonas hydrophila* isolated from gastroenteritis case in adult Iraqi male, on blood agar appear large, round, raised, opaque; it's not a halophilic microbe (don't growth in 6% NaCl).

Gram Negative Bacilli and CoccoBacilli (MacConkey –negative, Oxidase –Variable)

Genus;

Haemophilus

They are small, non- motile, non-sporing, gram negative rods or coccobacilli, most strains of *Haemophilus* spp. are able to grow aerobically and anaerobically, the addition of 5-10% CO₂ to the incubation atmosphere will enhance growth of many strains,oxidase variable, not grow on MacConkey agar, some strains need X and V to grow. Convex, smooth, pale gray and transparent with fishy smell, The colonies of capsulate strains are usually 0.5-1mm in diameter, circular, high convex in shape and mucoid. *Haemophilus influenzae* type b capsulated very important, cause meningitis in children, we can prevent by vaccine.

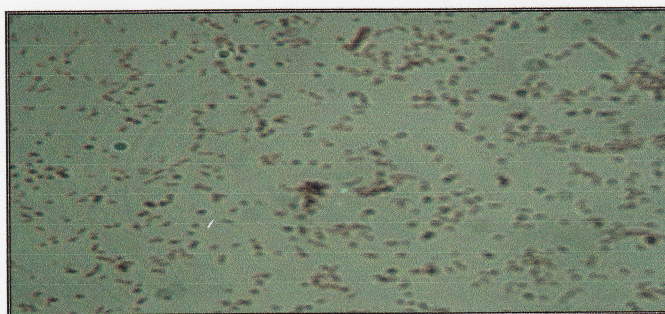


Figure (68): A gram stain film shows scattered Gram-negative coccobacilli faintly stained, small bacilli found to be *Haemophilus influenzae* type b, they yielded from a streak growth onto ch.A from the CSF sediment cultivation through 24 hours in 5% CO₂ at 35 °C.

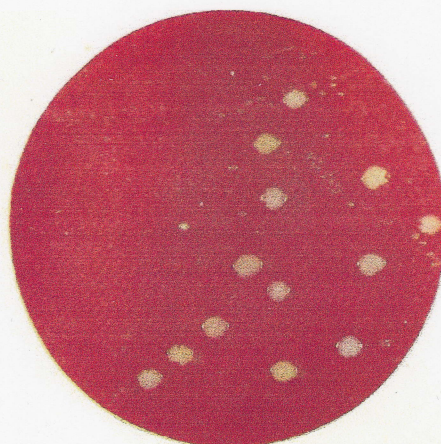


Figure (69) a growth of moist, smooth, gray colored colonies of *H.influenzae* isolated from a young Iraqi febrile male patient suspected to have PUO due to bacterial meningitis. We revealed that the microbe grows after 18-24 hours in 5% CO₂ at 35 °C, and we document our result via the pastor ax agglutination test, also we can notice the satellite phenomenon aggravated via *S.aureus* streaking perpendicular with the suspected microbe.

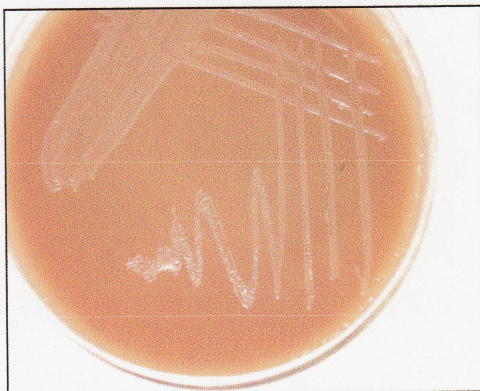


Figure (70) Clarified colonies of *Haemophilus influenzae* isolated from a sputum specimen cultivated onto chocolate agar plate under 2-3 % CO₂, notice the very small discrete gray-whitish colonies.

Table (7); Shows the required of X and V factor & hemolysis on blood agar to the different species of *Haemophilus*.

Species	Requires		Hemolysis
	X	V	
H influenzae (H aegyptius)	+	+	-
H parainfluenzae	-	+	-
H ducreyi	+	-	-
H haemolyticus	+	+	+
H parahaemolyticus	-	+	+
H aphrophilus	-	-	-
H paraphrophilus	-	+	-
H paraphrophaemolyticus	-	+	+
H segnis	-	+	-

Gram Negative bacilli that are optimally recovered on special media

Genus; **Brucella**

Small, nonmotile, aerobic, gram negative coccobacillary or short rods that stain poorly by conventional gram stain. Many isolates require supplementary carbon dioxide (CO₂) for growth, especially on primary isolation.

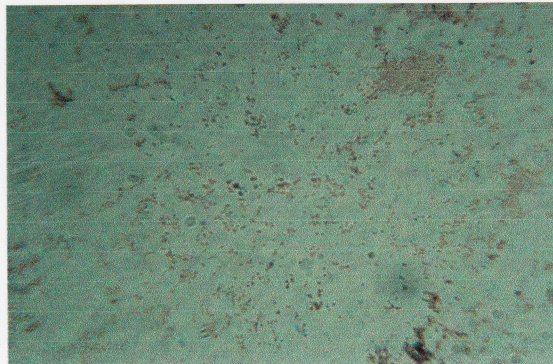


Figure (71) Clarified gram-negative coccobacilli which confused with *Bordetella*, *Moraxella*, *Kingella*, and *Acinetobacter* but it is *Brucella* gram film which is non-motile, urease (+), catalase (+), and oxidase (+).

The appearance in young cultures varies from cocci to rods, with short coccobacillary forms predominating; they are gram negative but often stain irregularly. on culture appear small,convex,smooth colonies, appear on enriched media in 2-5 days requires 5-10% CO₂,Brucella utilize carbohydrates but produce neither acid nor gas.Catalase and Oxidase are produced,H₂S is produced by many strains.

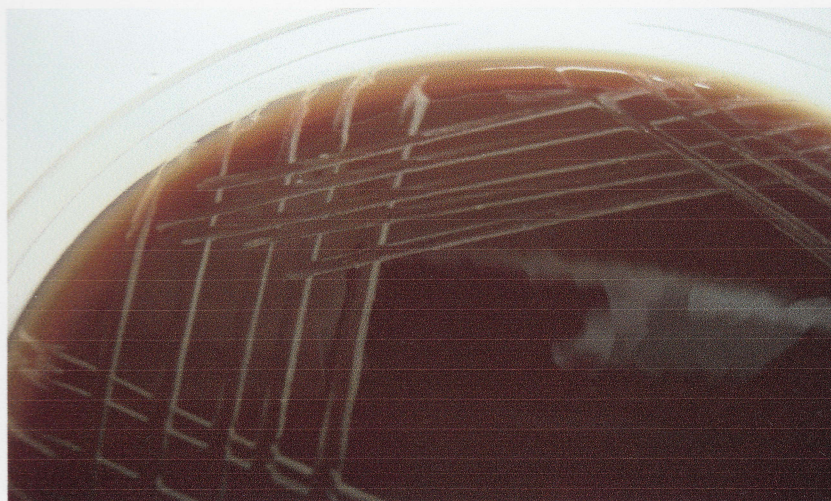


Figure (72) Revealed colonies of *Brucella abortus* isolated from febrile Iraqi adult female, the blood agar plate incubated at 5% CO₂ humidified incubator for 7 days, its colonies are small, convex, non-hemolytic, slight yellowish.

Culture; small, convex, smooth colonies appear on enriched media in 2-5 days
Requires 5-10% CO₂ for growth especially for *Brucella abortus*, *Brucella* utilizes carbohydrates but produce neither acid nor gas, Catalase and oxidase are produced. H₂S is produced by many strains.

A positive urease test is characteristic of *Brucella* species. *B. suis* and some strains of *B. melitensis* can yield a positive test less than 5 minutes after inoculating the slant; other strains will take a few hours to 24 hours.

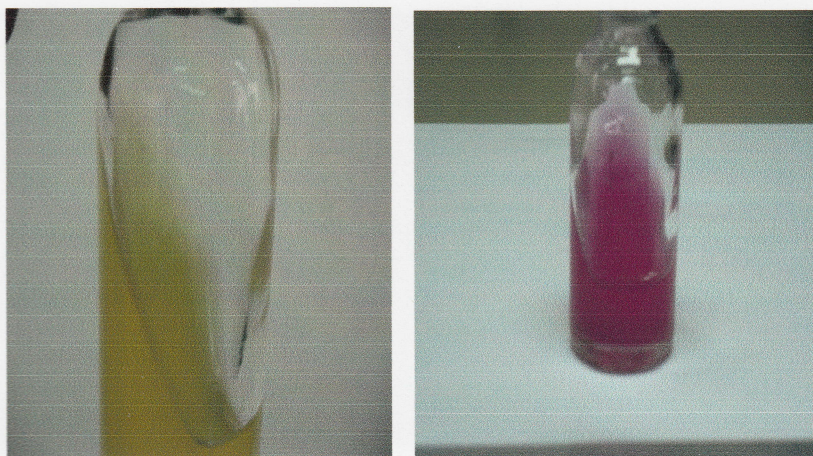


Figure (73) Shows the Christensen urea agar slant reactions regarding the *Brucella* species, notice its positive reaction (right); while the left slant is uncultivated.

Gram-negative cocci

Family: Neisseriaceae:

Genus;

Neisseria meningitides:

Oval gram negative diplococci, with flattened or concave opposing edges and the long axes parallel, typically seen in large numbers inside polymorph nuclear leucocytes or extracellular. Films from cultures show more rounded cocci and some polymorphism with irregular staining. Capsulated; non-sporing; non motile.

Cultural characters: Convex, gray and translucent after 48 h colonies are larger with an opaque raised centre and thin transparent margins, which may be, created no haemolysis on blood agar, Colonies are slightly larger on heated blood (chocolate) agar than on ordinary blood agar.

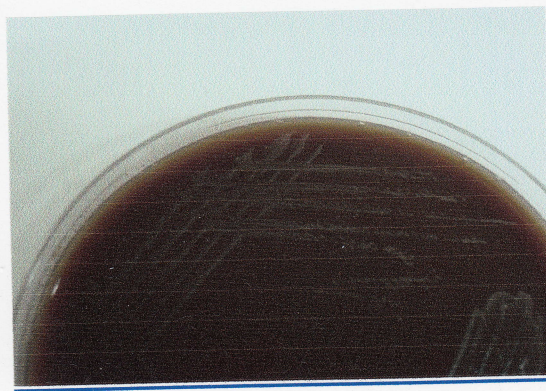


Figure (74): shows small, grayish white, convex, translucent, shiny colonies with either smooth margin to the *Neisseria gonorrhoeae*.

Neisseria gonorrhoeae:

Morphology and staining of *N. gonorrhoeae* are identical to those of *N. meningitidis*. The main character that distinguishes the gonococcus from the meningococcal is the ability to produce acid from glucose but not maltose.

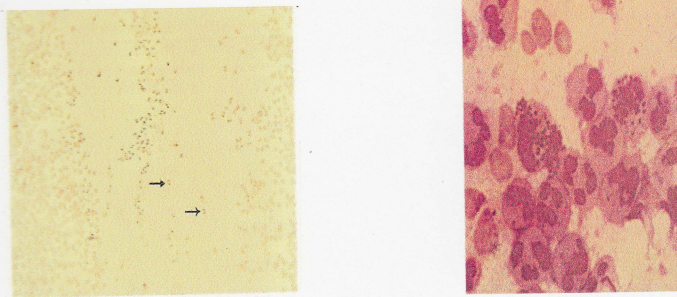


Figure (75): A Gram stained film of cervical swab. Notice the diplococci, which is the causative agent of gonorrhea. They are paired Gram-negative diplococci flattened to each other internally. (100X)

Genus; *Moraxella catarrhalis:*

Oxidase positive, inability to produce acid from sugars, non-motile.

Morphology and staining:

Oval gram-negative cocci single or in pairs with adjacent sides flattened.

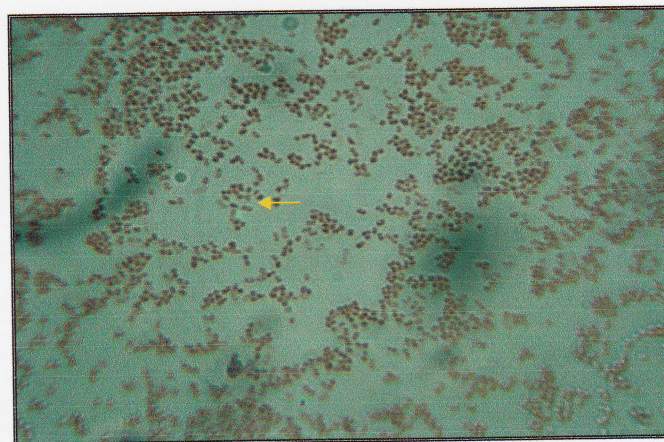


Figure (76): A Gram stained sputum film. Revealed the predominance of gram-negative diplococci (*Moraxella catarrhalis*), the etiology of different upper respiratory tract infections (URTIs) among human's (100X).

Cultural characters:

After incubation for 24 h, colonies on blood or heated blood agar is non-hemolytic, After 48 h colonies are larger, more elevated with a raised opaque center. Colonies are larger, more elevated with a raised opaque center white or grayish. Convex opaque center with an entire margin later becoming irregular.

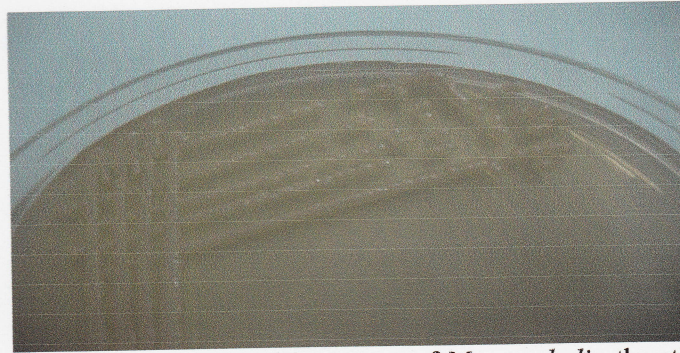


Figure (77): A streak plate onto Muller- Hinton agar of *M.catarrhalis*, the etiology incriminated here with acute bronchitis in 32 years old Iraqi male patient

Anaerobic, Gram- positive, Spore –forming Bacilli

Genus; *Clostridium*

Clostridium tetani; anaerobic, Gram positive slender, motile bacillus, the spore form has a characteristic drumstick or tennis-racket shape.

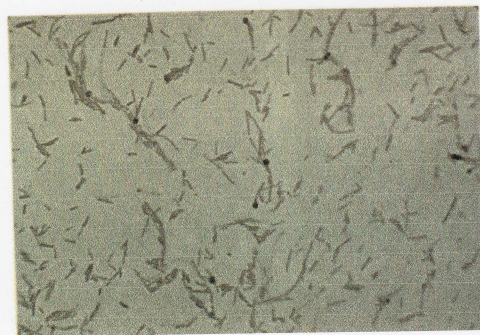


Figure (78) *Clostridium tetani* round terminal spores, the drumstick appearance .gram negative cells.

Clostridium botulinum; anaerobic, spore-forming bacilli often appears as gram positive in young cultures.



Clostridium perfringens; Gram positive, spore-forming, (spore may be either terminal or sub terminal, anaerobic rods, is relatively aero tolerant.



Figure (79) *Clostridium perfringens* boxcar-shaped cells

Clostridium perfringens can be recognized by its gray, spreading colonies, which often produce a double zone of hemolysis in blood agar, depending on the isolate and culture medium, an inner zone of complete clear hemolysis, and an outer zone of partial hemolysis.

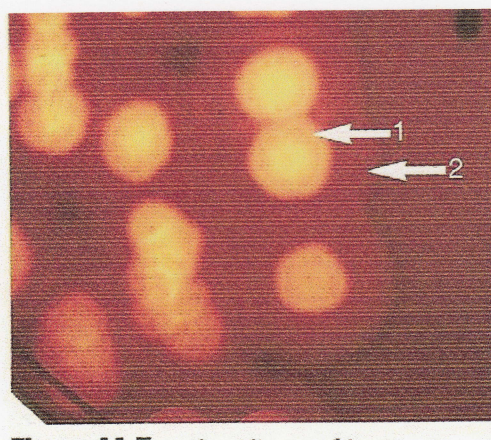


Figure (80) *Clostridium perfringens* on anaerobic blood agar .Note double zone of beta hemolysis.1.first zone; 2.second zone.

Genus;

Bacteroides:

Non motile, gram negative rod, often showing some pleomorphic, growing rapidly on anaerobic blood agar. After 48 hours the colonies are moderate circular and convex with a smooth gray or white appearance, colonies are non-hemolytic,

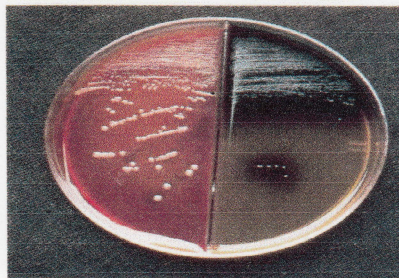
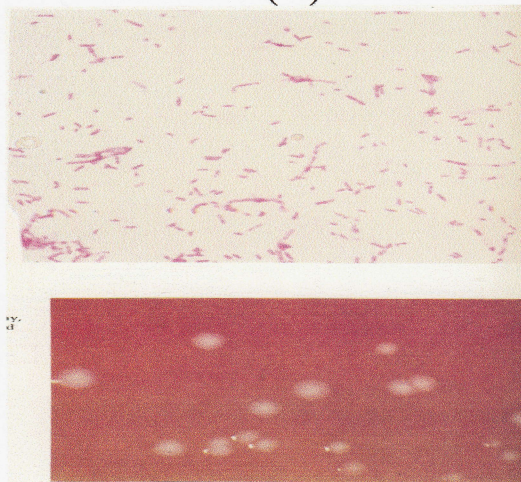


Figure (81) *Bacteroides fragilis* growing on bial plate of Bacteroides bile esculin and laked blood with kanamycin and vancomycin agar

(A)



(B)

Figure (82) *Bacteroides fragilis* .irregular staining and pleomorphism (A), gray, shiny colony on blood agar (B).

Mycobacteria

Genus;

Mycobacterium

Mycobacterium tuberculosis (T.B)

They are gram positive but many species poorly with this stain even after prolonged staining appear red bacilli by Ziehl Neelsen stain, straight or slightly curved rods, and non-motile, non-capsulate, non-sporing, strict aerobic, human infected by three spp., *Mycobacterium tuberculosis*, *Mycobacterium africanum* and *Mycobacterium bovis*, which are classified under major group of organisms called *Mycobacterium tuberculosis* complex, *Mycobacterium tuberculosis* acid and alcohol fast.

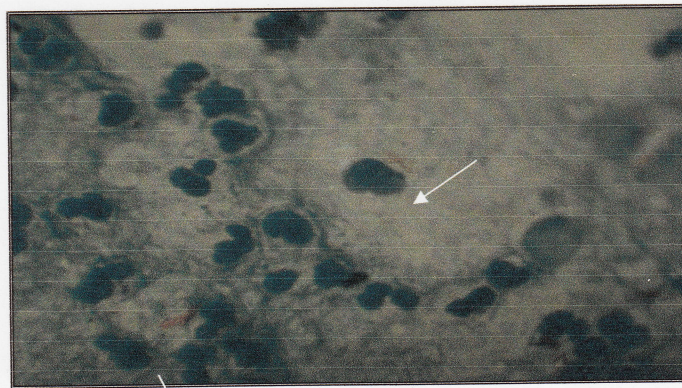


Figure (83): A Ziehl-Neelsen (Z.N.) sputum film obtained from 66 years old Iraqi male patient. Notice the red rods (acid fast bacilli)(*Mycobacterium tuberculosis*), plus bluish stained leukocytes (100X).



Figure (84): shows the acid fast stained rods (*M.tuberculosis*). Focus the cording phenomenon as they adhere together after cellular division due to high was content in their cell wall (100X).

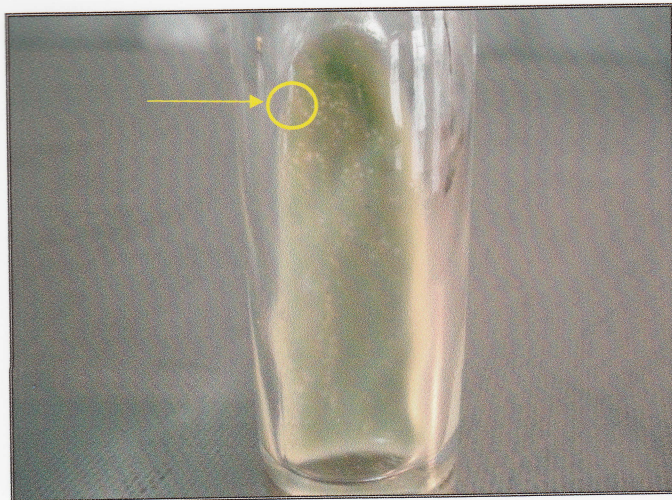


Figure (85): Shows the *Mycobacterium tuberculosis* growth onto inspissated egg yolk agar (Lowenstein-Jensen) agar slant. It grows slowly within 3-4 weeks, raised, rough, buff, tough colony.

Mycology

Genus;

Candida

Grow as oval, budding yeast cells, they also form pseudohyphae when the buds continue to grow but fail to detach, *Candida albicans* can be diagnostic by germ tube method to see chlamydospore, can produce true hyphae, on agar media produce soft, cream-colored colonies with a yeasty odor.

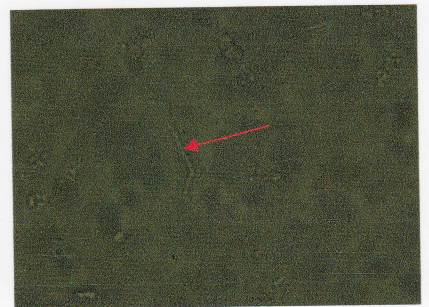
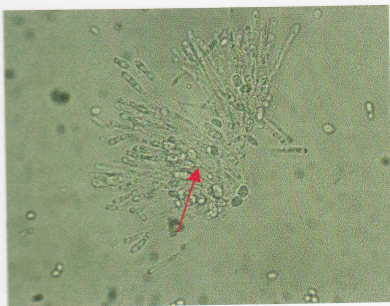


Figure (86): Clarified the physiological phenomenon of a germ tube formation by *C.albicans* regarding its incubation with Human serum for a half hour at 35 °C.

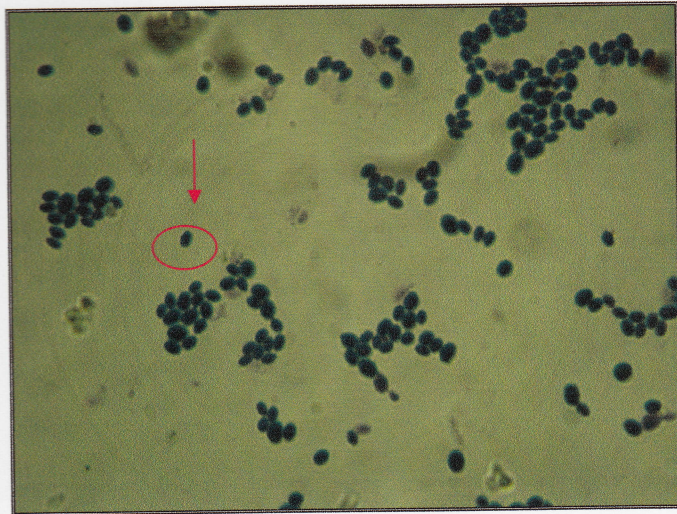


Figure (87): A Gram- film, clarified the Gram-positive yeast-like cells with budding which resembling *Candida albicans*, this result will be confirmed via the physiological tests (germ tube, and corn-meal agar plate growth for the chlamydospores) plus the biochemical tests (100X).

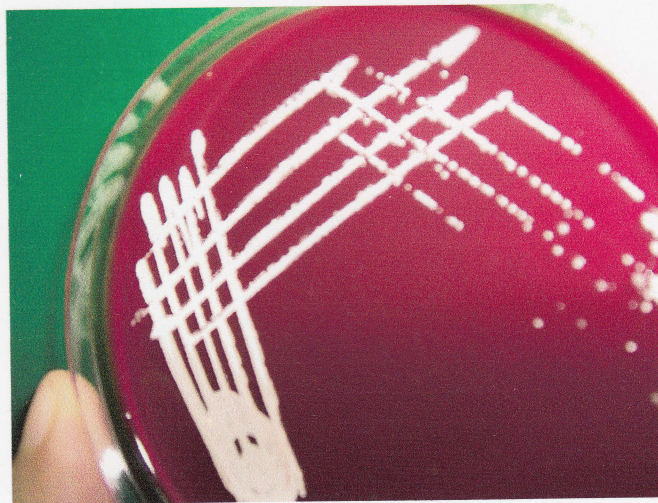


Figure (88): Shows the colonies of *C.albicans* grown onto blood agar plate; notice the smooth, opaque, creamy whitish, small-moderate colonies with its characteristic fermentation odor.

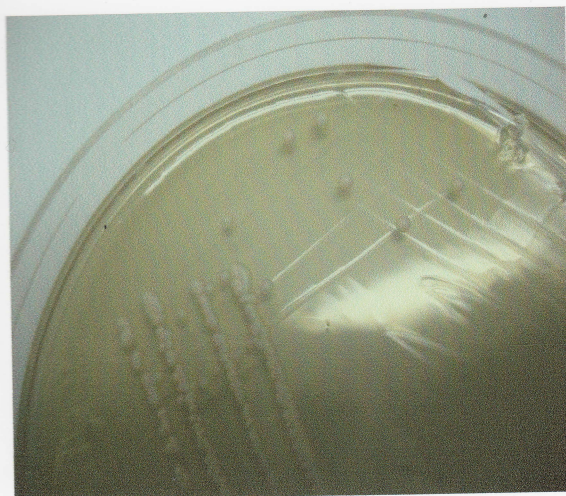


Figure (89): A growth of *Candida albicans* onto Sabouraud-dextrose agar plate which isolated from a case of right big toe onychomycosis in a diabetic adult Iraqi patient. Notice the whitish-creamy colonies, convex, entire, smooth, and opaque.

References:

1. Geo FB, Janet SB, Stephen AM. **Medical microbiology** 21st Edition (1998).
2. Gerald C, Barrie PM, Endrew GF, Barrie PM, Anthony S. **Practical Medical Microbiology** 40th Edition (1999).
3. Baily & Scott's. **Diagnostic Microbiology**. United States of America (1994).
4. R.R.Gillies and T.C.Dodds. **Bacteriology illustrated**. London (1973)
5. **Basic laboratory procedures in clinical bacteriology** World health organization (2003).
6. Baily & Scott's. **Diagnostic Microbiology**. United States of America (2002).
7. Baily & Scott's. **Diagnostic Microbiology**. United States of America (2007).